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The Spectroscopic Study of Biological Extracts

I. Urine*

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Increased interest in the metabolism of aromatic and cyclic hydrocarbons has made necessary the application of improved methods for the detection of minute amounts of these compounds in biological extracts. In metabolic studies only limited quantities of starting material are usually available and the amounts of certain metabolites present are too small to permit the isolation of pure compounds. Colorimetric, polarographic, fluorescence, and spectroscopic methods have been employed for the detection of these compounds, but the results have not been entirely satisfactory in all instances.

Spectroscopic procedures have been applied to the study of the constituents of biological materials, such as urine and blood (24, 26, 34, 39, 46, 55, 57, 63, 78, 101, 104), because many aromatic compounds isolated from these sources have specific absorption bands in the ultraviolet region of the spectrum. In most instances in the past, however, the spectroscopic studies have been carried out on unpurified material and the results have been somewhat unsatisfactory for the following reasons: (a) The materials studied contained a mixture of many aromatic compounds which gave overlapping absorption bands; hence, no specific and detailed bands suitable for the identification of substances in mixtures were observed. (b) The presence of a relatively large amount of a compound with a low extinction coefficient, or a small amount of a compound with a high extinction coefficient, interfered with the identification of other substances with a comparatively low extinction coefficient. (c) The presence of material responsible for a continuous absorption decreased the sharpness of individual bands and shifted them, thus making the interpretation difficult or uncertain.

Despite these disadvantages, spectroscopy provides in many instances more specific and detailed information in regard to the compounds under consideration than do colorimetric reactions. Because of the unsatisfactory results of previous work on untreated biological material (24, 26, 34, 39, 45, 46, 55, 57, 63, 78, 101, 104), a method of preliminary chemical fractionation of the ether-soluble substances of urines was employed by which the various absorbing compounds in a mixture could be separated from each other. At the outset it was necessary, therefore, to determine to what extent such fractionation procedures must be carried out in order to detect spectroscopically various aromatic substances in mixtures.

MATERIAL

Only studies on the urine of human beings are reported in this communication. Twenty-four hour specimens of urine were collected from 48 different individuals. Of these, 10 were normal, 26 were patients with pernicious anemia, refractory anemia, or leukemia, and 12 were pregnant women.[1] Several collections from the same individual were usually investigated.

METHODS

I. Spectroscopic Procedures

The spectograph was a small quartz instrument manufactured by Hilger (Model No. 484), taking a 3¼ by 4¼ inch plate. The light source employed was an all-quartz hydrogen discharge tube (manu-

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[1] The urine of pregnancy was obtained through the courtesy of Dr. Herbert Traut of the New York Hospital.

factured by the Hanovia Chemical Co.). In order to bring out all the absorption bands, a quartz Baly cell, with a micrometer adjustment, was employed. To compare the absorption bands of an unknown with those of a known substance, one plate was superimposed upon the other against a suitable light source. The hydrogen lines in the region of 4,800 A. were used as reference lines. A few plates were studied with a microphotometer and the tracings can be seen in Fig. 18. For the quantitative measurements a Spekker spectrophotometer with a condensed spark as the light source was used (Fig. 17).

II. Fractionation of Urine

Acidified urines, both hydrolyzed and unhydrolyzed, were extracted with ether,[2] and the ethereal extracts separated into fractions containing the acidic, phenolic, and neutral ether-soluble substances. The acidic and phenolic compounds were further separated by steam distillation into their steam-volatile and nonsteam-volatile components. An outline of the procedures of extraction and separation is presented in Fig. 1.

A. Preparation, hydrolysis, and ether extraction of urine (AE1 and BE1 fractions).—The urine excreted during a 24-hour period was collected with a few cc. of chloroform as a preservative and was kept in the refrigerator at a temperature of 4° C. The patients received no medication of any kind for 3 days before, as well as during, the collection period, since derivatives of certain drugs excreted in the urine may give absorption bands. Concentrated hydrochloric acid was added to the urine to bring the pH to about 1.0. The urine was subsequently divided into equal parts designated as A and B. Part A was extracted with ether without further treatment, and part B was hydrolyzed by autoclaving for 2 hours at a pressure of 15 pounds and a temperature of 115° C. (6, 14, 75, 89, 94, 97). Part B was then extracted with ether. In a number of experiments, the total acidified urine was extracted with ether. The urine residue was then hydrolyzed by autoclaving and was subsequently extracted with ether. Fractions extracted before hydrolysis are designated by the letters AE, while ethereal fractions from hydrolyzed urine are designated by the letters BE. Extractions were made continuously for 24 hours in a Kutscher-Steudel apparatus which provided an even distribution of the ether through a sintered glass disc. For the spectroscopic survey the ethereal fractions were concentrated on the steam bath to a volume of 10 to 50 cc.

[2] Mallinckrodt reagent ether was used. On several occasions it was noticed that after the ether had been concentrated 10 to 20 times a number of absorption bands were found. These were in the region of 2,700 A. and 3,000 A. For this reason it was necessary to examine each container of ether.

B. Separation of the ether-soluble substances into acidic, phenolic, and neutral components. 1. (a) Fraction containing the acidic substances (AE3 and BE3).—The ether extracts (AE1 and BE1) containing the total ether-soluble substances were brought to a volume of roughly 150 cc., and were extracted 3 times with 30 cc. of a solution of 10 per cent sodium carbonate. These combined sodium carbonate solutions were extracted 3 times with 150 cc. of ether, and all the ether extracts were combined to give the AE2 and BE2 fractions containing both the phenolic and the neutral substances. The sodium carbonate containing the acid substance was acidified with hydrochloric acid, extracted 3 times with equal volumes of ether, and the ethers combined to give the AE3 and BE3 fractions.

(b) Separation of the steam-volatile from the nonsteam-volatile acidic substances (AE8 and AE9, and BE8 and BE9).—Steam distillation was carried out in an all-glass apparatus of the Kjeldahl type fitted with an effective water-cooled condenser. The ether (AE3 and BE3) containing the acidic compounds was transferred to the Kjeldahl flask, 2 cc. of 10 per cent sodium hydroxide solution were added, and the ether was evaporated on the water bath. The fraction was then acidified with 5 cc. of 20 per cent hydrochloric acid solution, and steam-distilled until approximately 500 cc. of distillate was obtained, while the volume of the fluid in the flask was kept under 20 cc. Both the volatile material in the distillate and the nonvolatile material in the flask were made strongly acid with hydrochloric acid and extracted 3 times with equal volumes of ether. The ether extract containing the nonsteam-volatile material (AE8 and BE8) was concentrated on a steam bath to a volume of 10 cc., and that containing the steam-volatile material (AE9 and BE9) to a volume of 25 cc.

2. (a) Fraction containing the phenolic substances (AE5 and BE5).—The ether (AE2 and BE2) remaining after the sodium carbonate extraction was brought to a volume of approximately 150 cc. and was extracted 3 times with 30 cc. of 20 per cent sodium hydroxide solution. Subsequent steps were carried out exactly as has been described for the acid compounds. The final ethereal extract (AE5 and BE5) contained the phenolic compounds. The ether (AE4 and BE4) remaining after the sodium hydroxide extraction contained the neutral substances.

(b) Separation of the steam-volatile (AE7 and BE7) from the nonsteam-volatile (AE6 and BE6) phenolic compounds.—The fractions were prepared as described under B, 1(b).

3. Fraction containing the neutral substances (AE4 and BE4).—The ether remaining after extraction with sodium hydroxide (AE4 and BE4) was washed with

5 per cent hydrochloric acid and the ether was concentrated on a steam bath to a volume of 25 cc. This fraction contained the neutral substances.

complete separation of a mixture of the pure aromatic substances found in urine, as well as their spectroscopic differentiation, was necessary before any

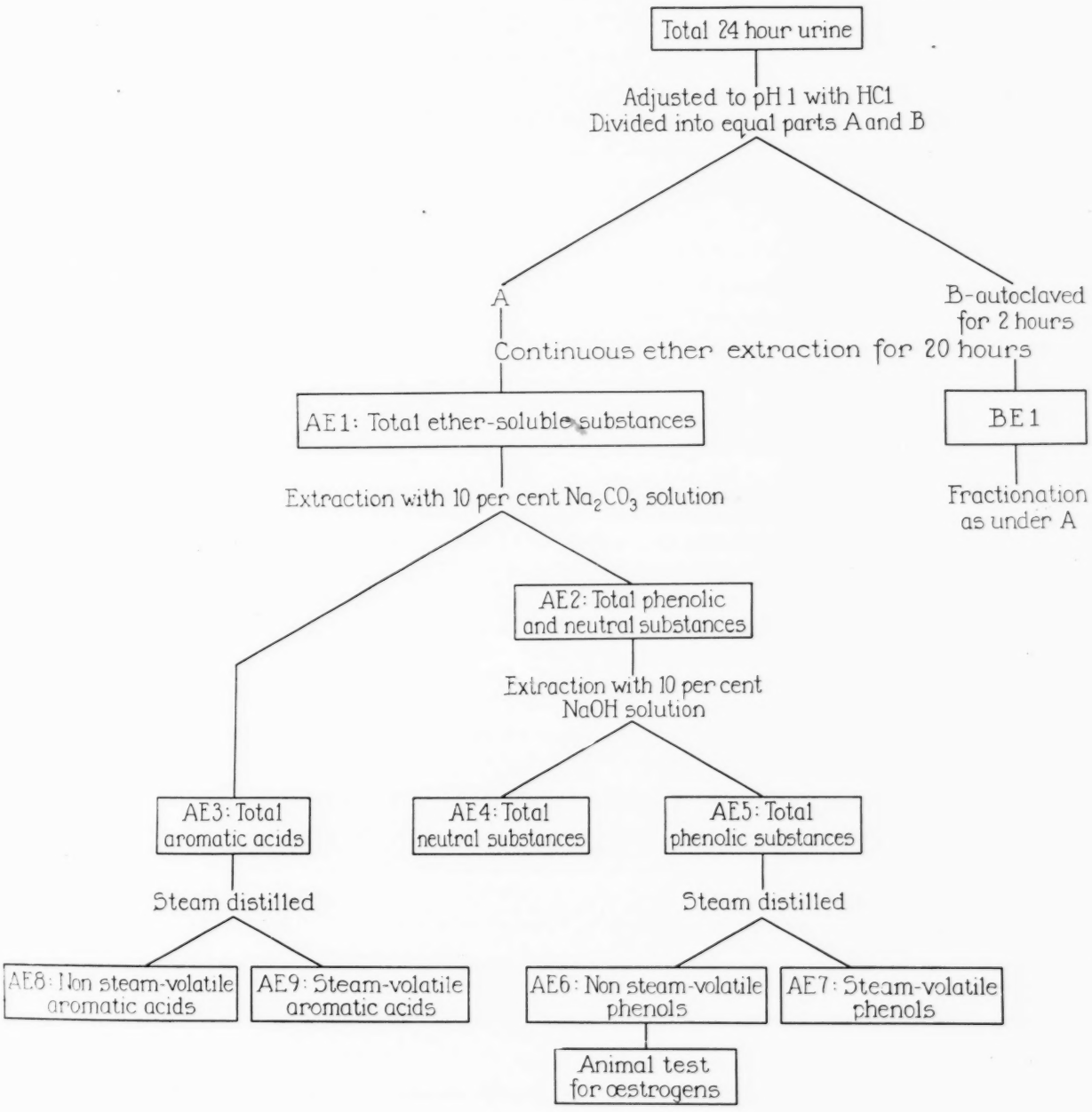


FIG. 1.—Scheme for the separation of the ether-soluble substances from urine.

RESULTS

A. EXPERIMENTS TO TEST THE COMPLETENESS OF THE FRACTIONATION PROCEDURE

A series of experiments with mixtures of pure chemical compounds was made to establish the adequacy of the method employed for fractionation. A spectroscopic survey of fractions of urine could be undertaken.

Experiment 1.—The first experiment was made to ascertain whether the steam-volatile, acidic, and phenolic compounds could be distilled quantitatively. Two different mixtures of steam-volatile substances, which are known to be present in urines, were worked up

as described under fractionation methods in II B. These mixtures were as follows: 1. mixture of benzoic acid (30 mgm.) and phenylacetic acid (70 mgm.); 2. mixture of phenol (10 mgm.) and *p*-cresol (90 mgm.).

The absorption bands given by the steam-volatile fraction were in the same positions as those given by the mixture of the pure substances. The fractions which should contain the nonsteam-volatile substances did not show any absorption bands, a fact which indicates that the distillation was complete as concerns both groups of substances.

Experiment 2.—A second experiment was made to test whether the nonsteam-volatile acidic and phenolic compounds would remain quantitatively in the nonsteam-volatile fraction. The following solutions containing pure substances were submitted to the fractionation procedure described under II B: 1. mixture of indolacetic acid (30 mgm.) and *p*-hydroxyphenylacetic acid (100 mgm.); 2. estriol (100 mgm.).

In the fraction which should contain the steam-volatile compounds no absorption bands were seen. In the fractions containing the nonsteam-volatile substances the absorption bands were in the same positions as the bands given by the pure compounds. Judged by the sensitivity of the spectroscopic method as applied, the separation was complete.

Experiment 3.—In a third experiment the following mixture, consisting of 8 pure neutral, phenolic, and acidic substances, was submitted to the complete chemical fractionation procedure as shown in Fig. 2: 1. neutral material,—indigo red (100 mgm.); 2. phenolic material,—phenol (10 mgm.), *p*-cresol (90 mgm.), estriol (100 mgm.); 3. acidic material,—benzoic acid (30 mgm.), phenylacetic acid (70 mgm.), indolacetic acid (30 mgm.), and *p*-hydroxyphenylacetic acid (100 mgm.).

As seen in Fig. 2, a separation of the compounds into fractions suitable for spectroscopic differentiation has been obtained. Identical solutions were made up artificially from pure compounds or mixtures of pure compounds. A comparison of the bands from the fractions and from the artificial solutions demonstrates that the bands given by the fractionated compounds or mixtures of compounds occur in the expected fraction and are identical with the bands of the artificial mixtures.

It can be seen from Fig. 2 that in fraction AE1, containing a mixture of 8 different substances, no differentiation of the individual components is possible. In fraction AE2, overlapping of bands prevents the differentiation of the 4 substances present. In fraction AE3, with 3 compounds present, the estriol cannot be differentiated. On the other hand, in fractions AE8, AE6, AE7, and AE4, absorption bands characteristic of all the 8 individual substances present in the mixture can be accounted for.

B. Spectroscopic Survey of Urines

1. General Discussion of the Ether-Soluble Aromatic Acids of Urine

Certain aromatic acids, products of the intermediate metabolism of aromatic amino acids and of the bacterial decomposition of proteins in the intestinal tract, are present in urine excreted by both normal and diseased individuals. The acids are all derivatives of phenylalanine, tyrosine, or tryptophane:

a. Phenylalanine derivatives:
   1. Benzoic acid (2, 22, 30, 47, 59, 76, 89, 96).
   2. Phenylacetic acid and phenylpropionic acid (1, 12, 31, 49, 76, 80, 82-84, 87-89, 98, 99, 102).
   3. Phenylpyruvic acid and phenyllactic acid (25, 38, 48, 52, 70, 77).

b. Tyrosine derivatives:
   1. *p*-Hydroxybenzoic acid (6, 14, 75, 89, 94, 97).
   2. *p*-Hydroxyphenylacetic acid and *p*-hydroxyphenylpropionic acid (2, 3, 5, 10, 11, 16, 17, 56, 58, 89-91, 97).
   3. *p*-Hydroxyphenylpyruvic acid and *p*-hydroxyphenyllactic acid (3, 17, 35, 40, 53, 54, 67, 95).

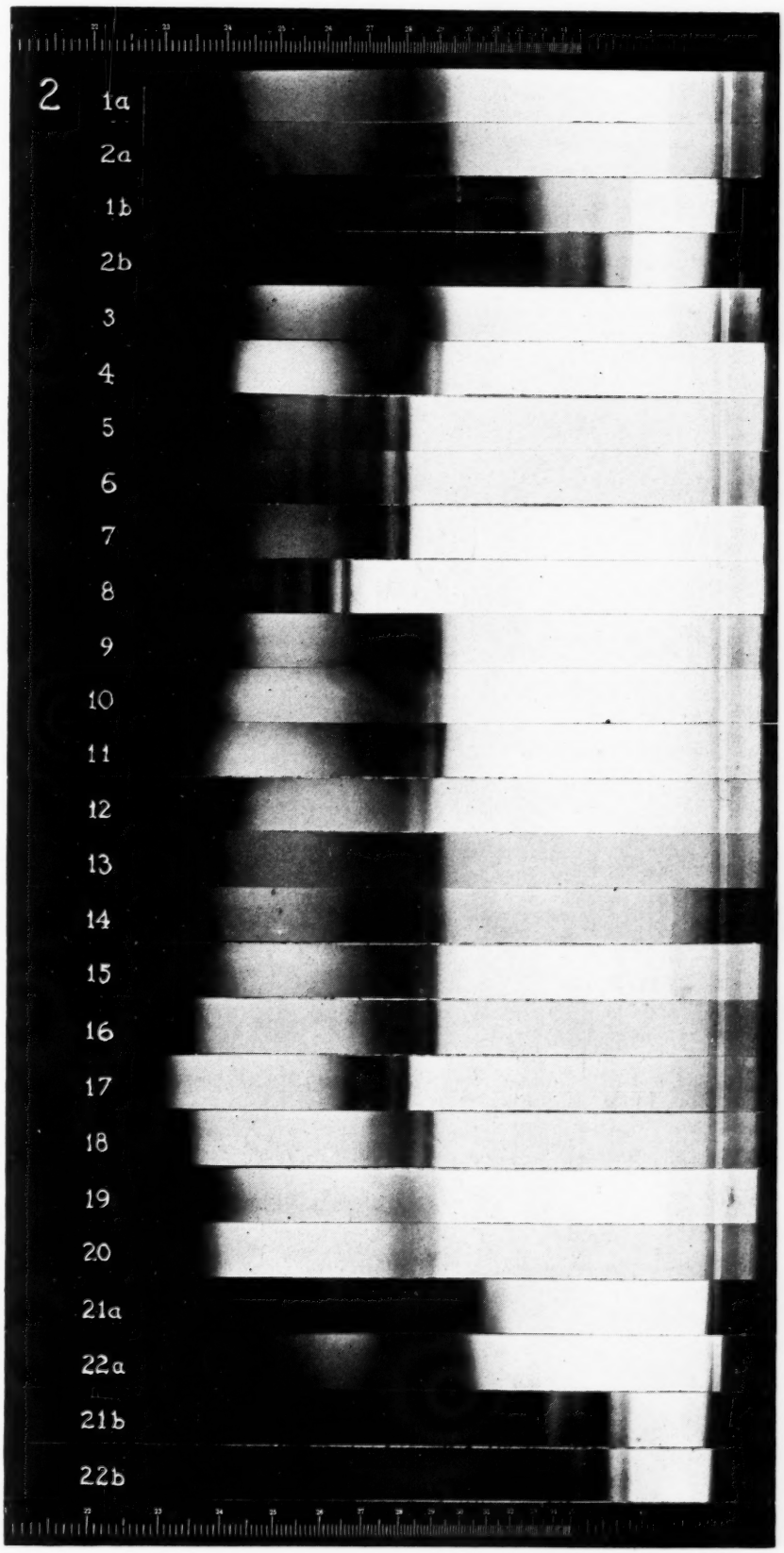
c. Tryptophane derivatives:
   1. Indol-3-acetic acid and indol-3-propionic acid (43, 44, 51, 85, 86).

A partial separation of these substances can be made by steam distillation, since the substances of groups

DESCRIPTION OF FIGURE 2

Fig. 2.—Absorption spectrograms of artificial mixtures of acidic, phenolic, and neutral aromatic substances.

(1a)—AE1, low concentration. (2a)—AE2, low concentration. (1b)—AE1, high concentration. (2b)—AE2, high concentration. (3)—AE3. (4)—Mixture of benzoic acid, phenylacetic acid, indolacetic acid, and *p*-hydroxyphenylacetic acid. (5)—AE9. (6)—Mixture of benzoic acid and phenylacetic acid. (7)—Benzoic acid. (8)—Phenylacetic acid. (9)—AE8. (10)—Mixture of indolacetic acid and *p*-hydroxyphenylacetic acid. (11)—Indolacetic acid. (12)—*p*-Hydroxyphenylacetic acid. (13)—AE5. (14)—Mixture of phenol, *p*-cresol, and estradiol. (15)—AE7. (16)—Mixture of phenol and *p*-cresol. (17)—Phenol. (18)—*p*-Cresol. (19)—AE6. (20)—Estradiol. (21a and b)—AE4. (22a and b)—Indigo red.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2a
1b
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18
19
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21a
22a
21b
22b

FIG. 2

a1 and a2 are steam-volatile while groups a3, b, and c are not steam-volatile.

The aromatic acids are present in urine partly in the free form but are principally conjugated with glucuronic acid, sulfate, glycocoll, or glutamine. The free compounds are readily soluble in ether, whereas the conjugated compounds are practically insoluble. The chemical isolation and identification of amino acid derivatives, with the exception of benzoic acid, is not practicable from 24-hour urine collections. Without actual isolation of compounds nonspecific tests must be employed (71). This is especially true for the oxidized aromatic compounds which give the same color reactions as do the phenols.

II. RESULTS OF SPECTROSCOPIC SURVEY OF THE ETHER-SOLUBLE AROMATIC ACIDS OF URINE

a. The total ether-soluble aromatic acids (Fig. 3) (AE3 and BE3).—This fraction gave a strong general absorption which decreased the intensity of the bands which were present. Furthermore, a mixture of substances with overlapping bands was present which made the differentiation of individual compounds difficult, if not impossible.

In (a) of Fig. 3 are shown the absorption bands given by the acidic ether-soluble substances from hydrolyzed urine (BE3); in (b) the bands given by the steam-volatile compounds of the same fraction (BE9); and in (c) the bands given by the nonsteam-volatile substances (BE8).

Comparison of the bands demonstrates that much sharper and more detailed bands were obtained after steam distillation since that procedure largely separated groups of compounds which gave overlapping absorption bands. A considerable amount of coloring material giving a background absorption remains in the solution after distillation, since it then becomes ether-insoluble.

b. Steam-volatile, ether-soluble aromatic acids. 1. Fraction obtained from unhydrolyzed urine.—Benzoic acid (AE9) Fig. 4. Absorption bands were present in this fraction (4a) at positions identical with those given by benzoic acid. This compound was liberated, presumably, by the hydrolysis of hippuric acid during the steam distillation of the (A) fraction. In many instances the bands given by this fraction from unhydrolyzed urine were identical with those given by a similar fraction obtained after hydrolysis, but the concentration of benzoic acid was greater in the latter.

For comparison the bands given by hippuric acid are shown in Fig. 4(c). These bands are similar to, but slightly different in position from those given by benzoic acid (b). The shift is so slight that it is only noticeable in the original plates and not in the reproductions.

2. Fraction obtained from hydrolyzed urine.—Benzoic acid and phenylacetic acid (BE9) Fig. 5. This fraction from urine (b) showed a series of bands similar to those given by a mixture of benzoic and phenylacetic acids (c). In Fig. 5a the absorption of benzoic acid and in Fig. 5d that of phenylacetic acid is shown. In the urines of normal, as well as of diseased individuals, there were considerable variations in the relative amounts of these compounds.

Although both phenylacetic acid and phenylpropionic acid may occasionally be present in urine (1, 12, 31, 49, 76, 80, 82-84, 87-89, 98, 99, 102), one cannot differentiate, spectroscopically, the latter (Fig. 6c) in the presence of the former (Fig. 6a) because of the slight difference in the absorption of these homologues. In illustration of this point, the absorption bands of pure phenylpropionic acid alone are compared with those of a mixture of pure phenylpropionic acid and phenylacetic acid (Fig. 6b and 6c). In the urines so far investigated, the absorption bands characteristic of phenylpyruvic (Fig. 6h) and phenyllactic acids have not been observed. The bands given by some of these acids and their mixtures are shown in Fig. 6.

c. The nonsteam-volatile, ether-soluble, aromatic acids (AE8 and BE8). 1. Indol-3-acetic acid (Fig. 7).[3]

[3] Most of the photographs shown in this paper are plates of enlarged two diameters. Since the details of the characteristic bands of indolacetic acid are rapidly lost on enlargement, contact prints had to be used in this instance.

DESCRIPTION OF FIGURES 3 TO 9

FIG. 3.—(a)—Total aromatic acids from hydrolyzed urine (BE3). (b)—Steam-volatile constituents from (a) (BE9). (c)—Nonsteam-volatile constituents from (a) (BE8).

FIG. 4.—(a)—Steam-volatile aromatic acids from unhydrolyzed urine (AE9). (b)—Benzoic acid. (c)—Hippuric acid.

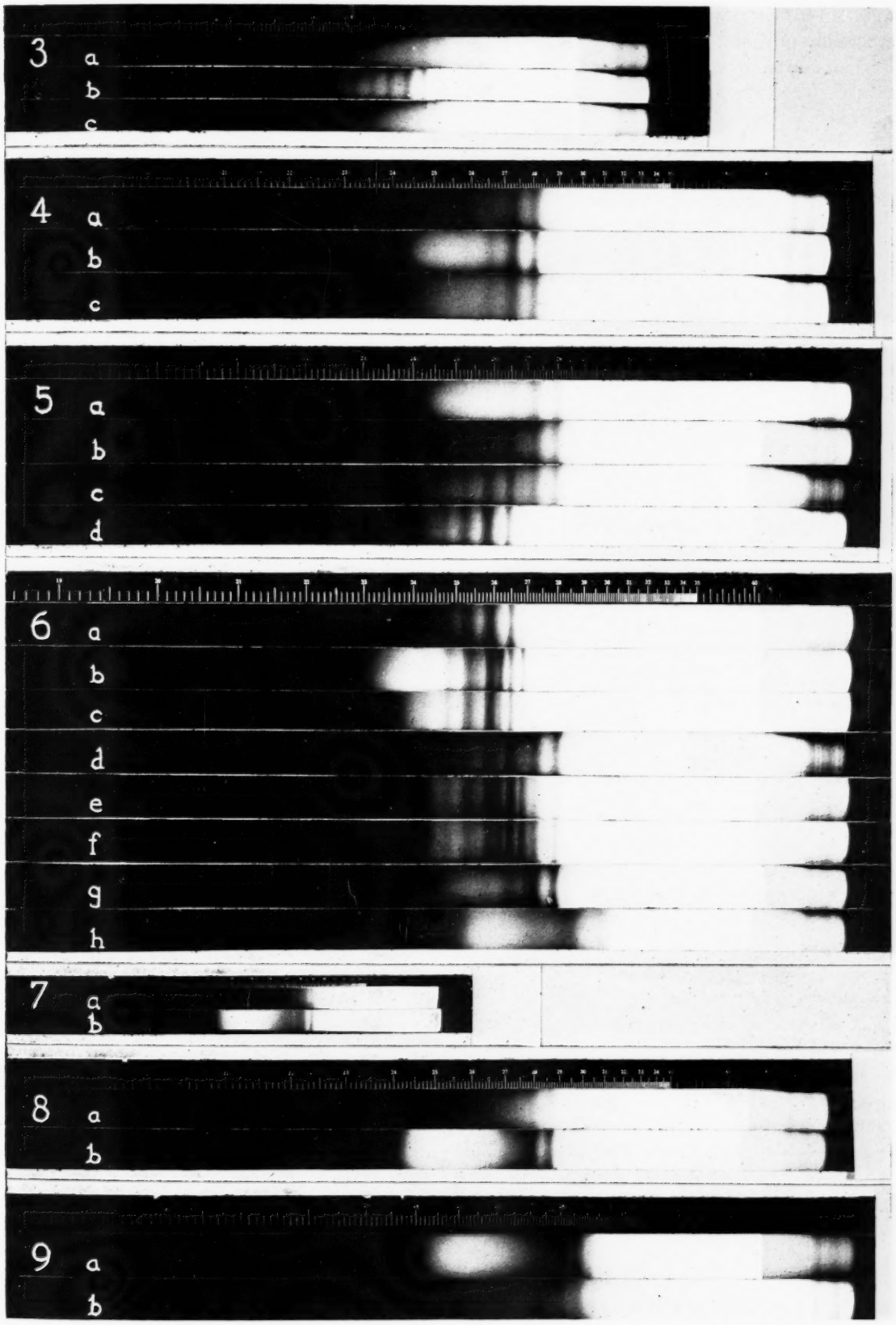
FIG. 5.—(a)—Benzoic acid. (b)—Steam-volatile aromatic acids from hydrolyzed urine (BE9). (c)—Mixture of benzoic and phenylacetic acids. (d)—Phenylacetic acid.

FIG. 6.—(a)—Phenylacetic acid. (b)—Phenylpropionic acid. (c)—Mixture of phenylacetic acid and phenylpropionic acid (1:1). (d)—Mixture of phenylacetic acid, phenylpropionic acid, and benzoic acid (3 parts of a mixture of 1:1 phenylacetic and phenylpropionic acid and 1 part of benzoic acid). (e)—Mixture of benzoic and phenylpropionic acids (1:3). (f)—Mixture of benzoic and phenylacetic acid (1:3). (g)—Benzoic acid. (h)—Phenylpyruvic acid.

FIG. 7.—(a)—Nonsteam-volatile aromatic acids from unhydrolyzed urine (AE8). (b)—Indol-3-acetic acid.

FIG. 8.—(a)—Nonsteam-volatile aromatic acids from hydrolyzed urine (BE8). (b)—*p*-Hydroxyphenylacetic acid.

FIG. 9.—(a)—Mixture of indol-3-acetic and *p*-hydroxyphenylacetic acids. (b)—Nonsteam-volatile aromatic acids from unhydrolyzed urine (AE8).



FIGS. 3 TO 9

—In fractions of urines from both healthy and diseased individuals bands were occasionally present in positions similar to those of indol-3-acetic acid (Fig. 7). The bands were present only in the fractions from unhydrolyzed urine, since acid hydrolysis transforms indol derivatives.

Indol-3-propionic acid gives the same series of bands as indol-3-acetic acid except that the bands are slightly shifted towards the longer wave lengths. Spectroscopic differentiation of these two compounds is not possible, because of the similarity in the absorptions given by these two homologues.

2. *p-Hydroxyphenylacetic acid* (*Fig. 8*).—Extracts from a few normal as well as from pathological urines, chiefly after hydrolysis, gave the bands illustrated in Fig. 8a. These are in the same position as those given by *p*-hydroxyphenylacetic acid (8b).

No statement can be made at this time concerning the possible presence of *p*-hydroxybenzoic acid and *p*-hydroxyphenylpropionic acid. However, there was no spectroscopic suggestion for the presence of *p*-hydroxyphenyllactic acid and *p*-hydroxyphenylpyruvic acid in the urine investigated.

If indol-3-acetic and *p*-hydroxyphenylacetic acids are present in the same solution, both can be detected, as seen in Fig. 9. The narrow band in the region 2,920 A., given by the indolacetic acid in the mixture of indolacetic and *p*-hydroxyphenylacetic acids, can also be seen in the original plate; it does not show plainly in the enlargement.

III. GENERAL DISCUSSION OF THE ETHER-SOLUBLE PHENOLS OF URINE

The presence of the following three groups of phenolic compounds in urine is well established:

Group 1. The monophenols,—phenol, *p*-cresol, and possibly *o*- and *m*-cresol (3, 4, 6-8, 13, 16, 19-21, 33, 36, 37, 41, 42, 69, 73, 81, 100, 103).

Group 2. The diphenols,—catechol, and possibly hydroquinone (3, 4, 8, 9, 15, 66, 74, 92).

Group 3. The estrogenic phenols,—estrone, estriol, and estradiol (23, 27-29, 32, 60-62, 68).

The monophenols (group 1) can be easily steam-distilled, the diphenols (group 2) are very slightly steam-volatile, and the estrogenic phenols (group 3) are nonsteam-volatile. This difference makes possible the separation of group 1 from groups 2 and 3.

Those substances are present principally in their conjugated forms as glucuronides, ethereal sulfates, or both. Very little is known about the ratio of free to conjugated phenolic compounds in urine under either normal or pathological conditions. The conjugated compounds are either slightly soluble or insoluble in ether in contrast to the free substances which dissolve easily in that solvent. The conjugated phenol and *p*-cresol hydrolyze readily in the cold with mineral acids (68), whereas the conjugated estrogenic compounds split in the presence of mineral acids only after heating.

The isolation and identification by chemical methods of the very small amounts of phenolic compounds present in the urine in 24 hours is very difficult. For the identification of these substances color tests such as the Millon or the Folin-Denis reactions frequently are used, but they are not sufficiently specific to allow differentiation of the phenols (18, 27, 50, 64, 71, 72, 79, 93, 105). For clinical purposes the estrogenic phenols are determined either by biological methods or by the Kober reaction.

IV. RESULTS OF SPECTROSCOPIC SURVEY OF THE ETHER-SOLUBLE PHENOLS OF URINE

a. The fraction containing the total ether-soluble phenolic material (*AE5 and BE5*).—The bands given by this fraction are in positions identical with those given by a mixture of *p*-cresol and phenol. The bands are very often decreased in their intensity by materials which give background absorption. The identification of the estrogenic phenols and diphenols in this fraction is not possible on account of the relatively high concentration of the *p*-cresol and phenol which give absorption bands in about the same region of the spectrum.

In most of these fractions obtained before, as well as after, hydrolysis, additional bands similar in position to those given by a solution of indigo blue were present in the region of 2,420 A. and 2,480 A. This compound was presumably produced from indoxyl by the fractionation procedure (18, 64, 72, 79, 105), and this will be discussed below.

b. The steam-volatile phenolic compounds p-cresol and phenol (*AE7 and BE7*).—The absorption bands of these fractions were the same as those described under a., except that the bands of indigo blue were not present. So far only *p*-cresol and phenol have been detected spectroscopically in this fraction (Fig. 10). *p*-Cresol is present in larger amounts than phenol in the extract from both unhydrolyzed and hydrolyzed urines. In different specimens the relative concentrations of these two substances varied, and both were present in increased amounts after hydrolysis. No information is at hand to establish whether the *p*-cresol and phenol were present in free form in the urine before treatment, or were produced during the continuous ether extraction by acid hydrolysis of a conjugated compound at room temperature (68).

In Fig. 11 are shown the absorption spectra of the isomeric, ortho-, meta-, and para-cresols, and it is apparent that para-cresol can be differentiated from the ortho and meta compounds. It is reasonable to

believe that if a great excess of phenol were not present, mixtures of phenol and para-cresol could be distinguished from mixtures containing phenol and meta-cresol, or phenol and ortho-cresol. It would be very difficult to distinguish between mixtures of phenol and ortho-cresol or phenol and meta-cresol.

c. The nonsteam-volatile phenolic compounds (AE6 and BE6).—1. The estrogenic phenols (Fig. 13). This fraction contained the phenolic female sex hormones and was of particular interest. The urines of normal adults of both sexes, as well as of twelve late pregnancies, were examined before and after hydrol-

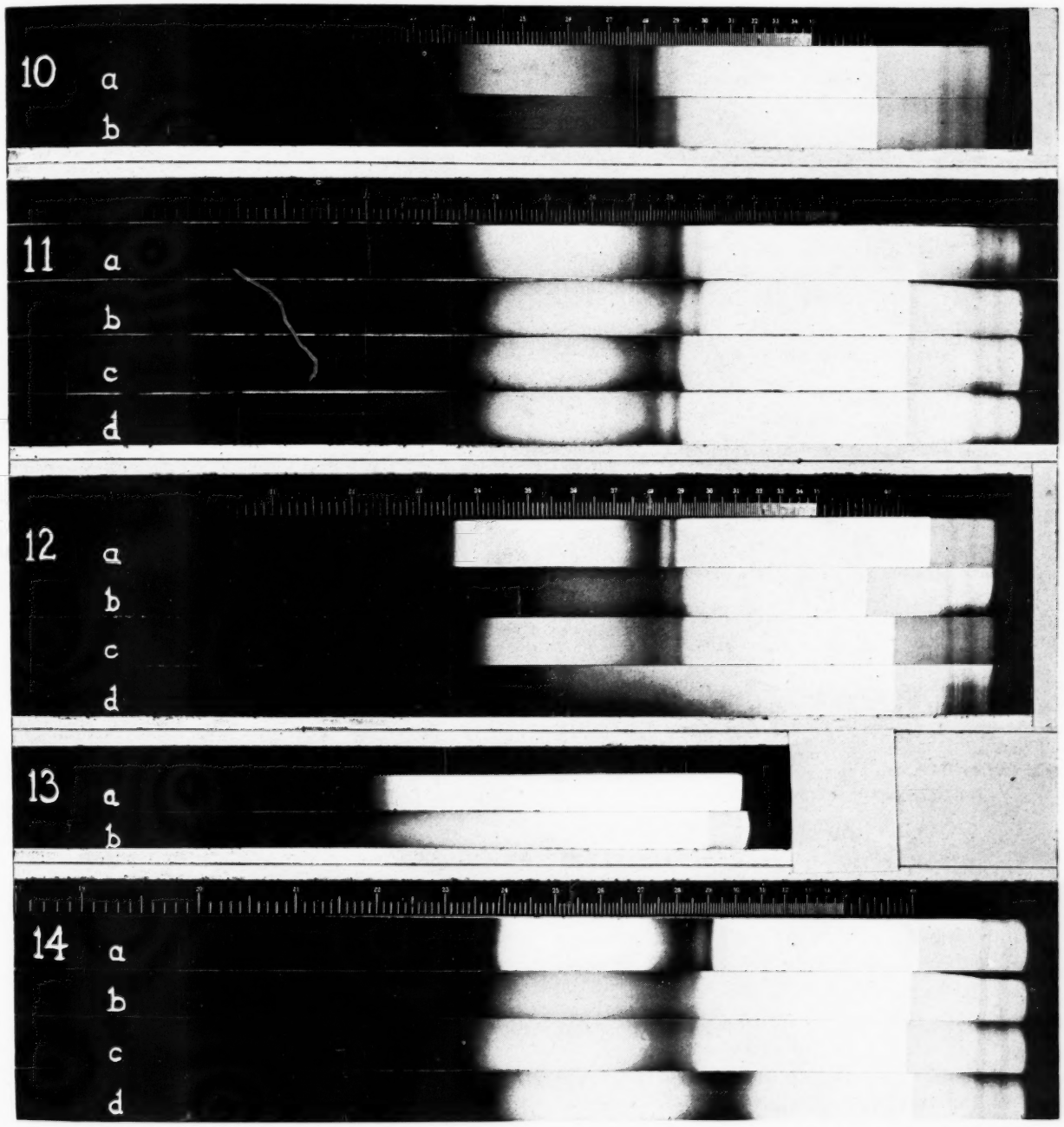


Fig. 10.—(a)—Mixture of *p*-cresol and phenol. (b)—Steam-volatile phenolic compounds from unhydrolyzed urine (AE7).

Fig. 11.—(a)—Mixture of *p*-cresol and phenol. (b)—*p*-Cresol. (c)—*o*-Cresol. (d)—*m*-Cresol.

Fig. 12.—(a)—*p*-Cresol. (b)—Nonsteam-volatile phenolic compounds from hydrolyzed urine (late pregnancy) (AE6). (c)—Estrone. (d)—Nonsteam-volatile phenolic compounds from hydrolyzed urine (normal female) (AE6).

Fig. 13.—(a)—Indigo blue. (b)—Absorption given by fraction AE6.

Fig. 14.—(a)—Estriol. (b)—Catechol. (c)—Nonsteam-volatile phenols from unhydrolyzed urine (pernicious anemia) (AE6). (d)—Hydroquinone.

ysis. No absorption bands similar to those of the estrogenic phenols were given by extracts of 24-hour urine collections of normal individuals, or of unhydrolyzed urines of pregnant women. In fractions from the hydrolyzed urines of late pregnancy which contain relatively large amounts of estrogenic material, absorption bands in positions identical with those of estrogenic phenols were found (Fig. 12b). The same fractions were tested for biological activity in oophorectomized rats and were strongly estrogenic. No absorption bands (Fig. 12d) and no positive biological reactions were obtained with similar fractions of urine from nonpregnant women.

A spectroscopic differentiation between estrone, estradiol, and estriol in this fraction is not possible without further chemical separation (23, 27-29, 32, 60-62, 68), since these substances give the same types of band structure in approximately the same positions (45).

the positions of the bands are different, these compounds can be easily differentiated, as seen in Fig. 14. Hydroquinone, if present, would absorb in a different region of the spectrum, the maximum being about 3,000 A. (Fig. 14d).

For investigations concerning the presence of these diphenols in the urine, the fractionation procedure should be carried out in the presence of a reducing agent (sodium hydrosulfite) because the diphenols are easily oxidized and undergo rapid alteration in strongly alkaline solutions.

V. THE ETHER-SOLUBLE, NEUTRAL COMPOUNDS OF URINE (FIG. 15)—GENERAL DISCUSSION AND RESULTS

Among the neutral aromatic compounds giving absorption in the ultraviolet region of the spectrum, indigo red has been isolated from urines by a number of investigators (18, 64, 72, 79, 105). The ether extracts of unhydrolyzed urine regularly were of a bright

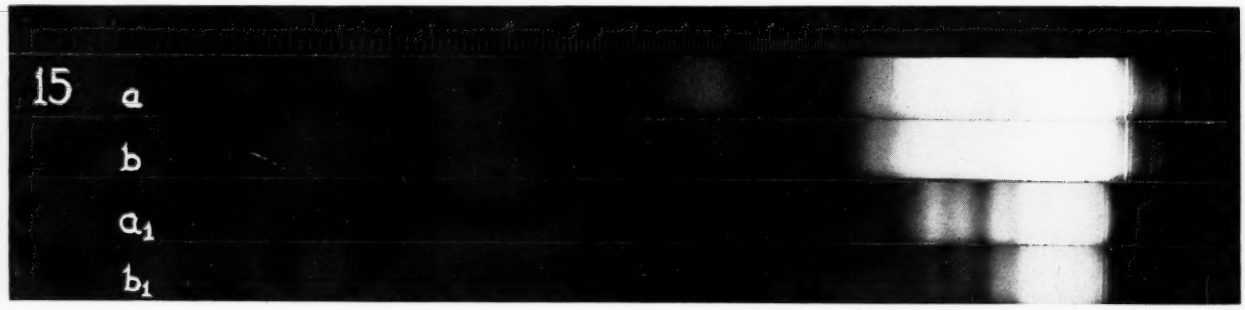


FIG. 15.—(a)—Indigo red in low concentration to show the short wave length band. (a_1)—Indigo red in higher concentration to show the longer wave bands. (b)—Neutral compounds from unhydrolyzed urine in low concentration (AE4). (b_1)—Neutral compounds from unhydrolyzed urine in higher concentration (AE4).

2. Indigo blue (Fig. 13). When the fraction containing the estrogenic phenols was diluted, two bands in the region of 2,420 A. and 2,480 A. usually were found in positions similar to the bands given by indigo blue. An ethereal solution of indigo blue also gives a weak band at about 2,820 A. which is usually too faint to be seen in the urine extract.

Indigo blue, as well as indigo red (18, 64, 72, 79, 105), is formed from its precursor, indoxyl, in the course of the treatment of the urine with mineral acids. The nonsteam-volatile indigo blue, only slightly soluble in ether, was partly extracted from the urine fraction.

3. Diphenols: catechol and hydroquinone (Fig. 14). Catechol and hydroquinone, if present in the urine, are found in the fraction containing the nonsteam-volatile phenolic compounds. In one case of pernicious anemia in relapse the fraction from unhydrolyzed urine gave bands similar to those given by catechol (Fig. 14c). These bands are in the same general region as those given by the estrogens, but because

reddish-purple color, but this was only rarely the case when the urine had been hydrolyzed before extraction. The intensity of the color varied in the extracts of urine from different individuals. When studied spectroscopically, this fraction gave a series of bands as seen in Fig. 15. Bands in similar positions were given by ethereal solutions of indigo red,[4] a substance formed by the oxidation of indoxyl in alkaline urine (18, 64, 72, 79, 105). In acid urine a similar reaction is caused by mild oxidizing agents. As a result of the same process, indigo blue also is formed, but that compound is only slightly soluble in ether, whereas indigo red is more readily dissolved.

VI. COMPARISON OF RESULTS OBTAINED BY VARIOUS SPECTROSCOPIC TECHNICS

In Fig. 16, the spectra of an ethereal solution of pure phenol (A), *p*-cresol (B), and their mixture (C)

[4] The indigo red was synthesized by Dr. Brooker and was obtained through the courtesy of Dr. Lloyd A. Jones, Eastman Kodak Company, Rochester, N. Y.

are shown. A continuous light source was used. For comparison, the curves of these substances and their mixture obtained by means of a Spekker spectrophotometer are shown in Fig. 17. A spark was used as a source of light. In Fig. 17, D represents the absorption curve of a urinary fraction containing the steam-volatile phenols. The maxima and minima of this fraction are influenced by the background absorption of some material of unknown nature.

In Fig. 18, a comparison is made of the band spectra and their corresponding photometric tracing [5] of the following materials: (A) the total ether-soluble phenols from urine; (B) the nonsteam-volatile fraction of A; (C) the steam-volatile phenols from A; and (D) a mixture of pure phenol and *p*-cresol.

DISCUSSION

The use of a continuous light source in the spectroscopic study of fractionated urinary extracts results in the appearance of well-defined bands, or groups of bands, which can be compared directly with those given by one reference compound or by a mixture of reference compounds. For the purpose of a qualitative survey, the spectroscopic method has been found rapid and satisfactory. This point is supported by a recent publication of Müller and Scholtan (65). Although the method is not a substitute for the standard quantitative methods of spectrophotometry, the qualitative procedure yields roughly quantitative information.

Since the fractions of urine are uniformly prepared, and since the spectroscopic work is done under standard conditions, the intensity of the bands from a fraction of urine can be compared with those given by reference solutions of known concentration, and thus a rough indication can be gained of the amount present. The application of standard quantitative spectroscopic methods to fractions of urine is hindered to some extent, however, by the presence of background absorbing material, which persists even after the preliminary chemical fractionation herein described.

The chemical methods of fractionation are the conventional procedures employed for the separation of acidic, phenolic, and neutral substances from organic solvents. A separation of the aromatic compounds was necessary in order to overcome the difficulties encountered in previous work in which it had been shown that spectroscopic examination of untreated materials did not show sufficient resolution for the detection of specific compounds. These disadvantages have been eliminated to a considerable extent by the present method of fractionation, but still further development of the chemical procedures is desirable. The aromatic bases and nonether-soluble aromatic compounds remain to be investigated.

The completeness of the chemical separation procedure may be inferred from the results of a similar fractionation of a mixture of pure aromatic acidic, phenolic, and neutral compounds which had previously been reported by other workers to be present in urines. From the presence of groups of bands in the proper chemical fractions and the absence of these bands in all of the other fractions, it may be inferred that the preliminary separation procedure was satisfactory. The same control was applied to all fractions obtained from urines, and on this basis, a second fractionation was necessary only occasionally. Within the limit of the sensitivity of the spectroscopic methods used here, all separations described in this paper were satisfactorily complete.

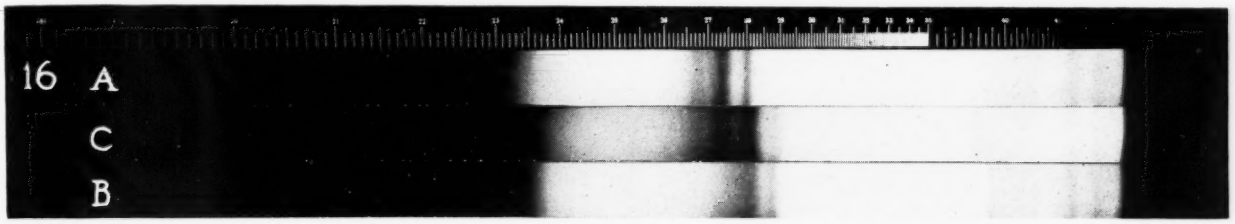


FIG. 16.—(A)—Phenol. (B)—*p*-Cresol. (C)—Mixture of phenol and *p*-cresol.

As shown in the results, the detection of various aromatic compounds by spectroscopic methods has been facilitated greatly by the use of fractionation procedures. However, the application of the method is limited for the following reasons: (a) Nonspecific, continuously absorbing material may blur or conceal the discrete absorption bands of substances present in low concentration. (b) The presence of compounds possessing absorption bands with high extinction coefficients will prevent the resolution of the bands of substances characterized by a low extinction coefficient. (c) The proximity of bands from two or three substances may cause overlapping to an appreciable extent, thus preventing identification. As an extreme case, overlapping can be complete, particularly in the case of such homologues as phenylacetic acid and phenylpropionic acid, both of which have been iso-

[5] We are indebted to Dr. E. G. Pickels of the International Health Division, Rockefeller Institute for Medical Research, for the photoelectric tracings of the plates.

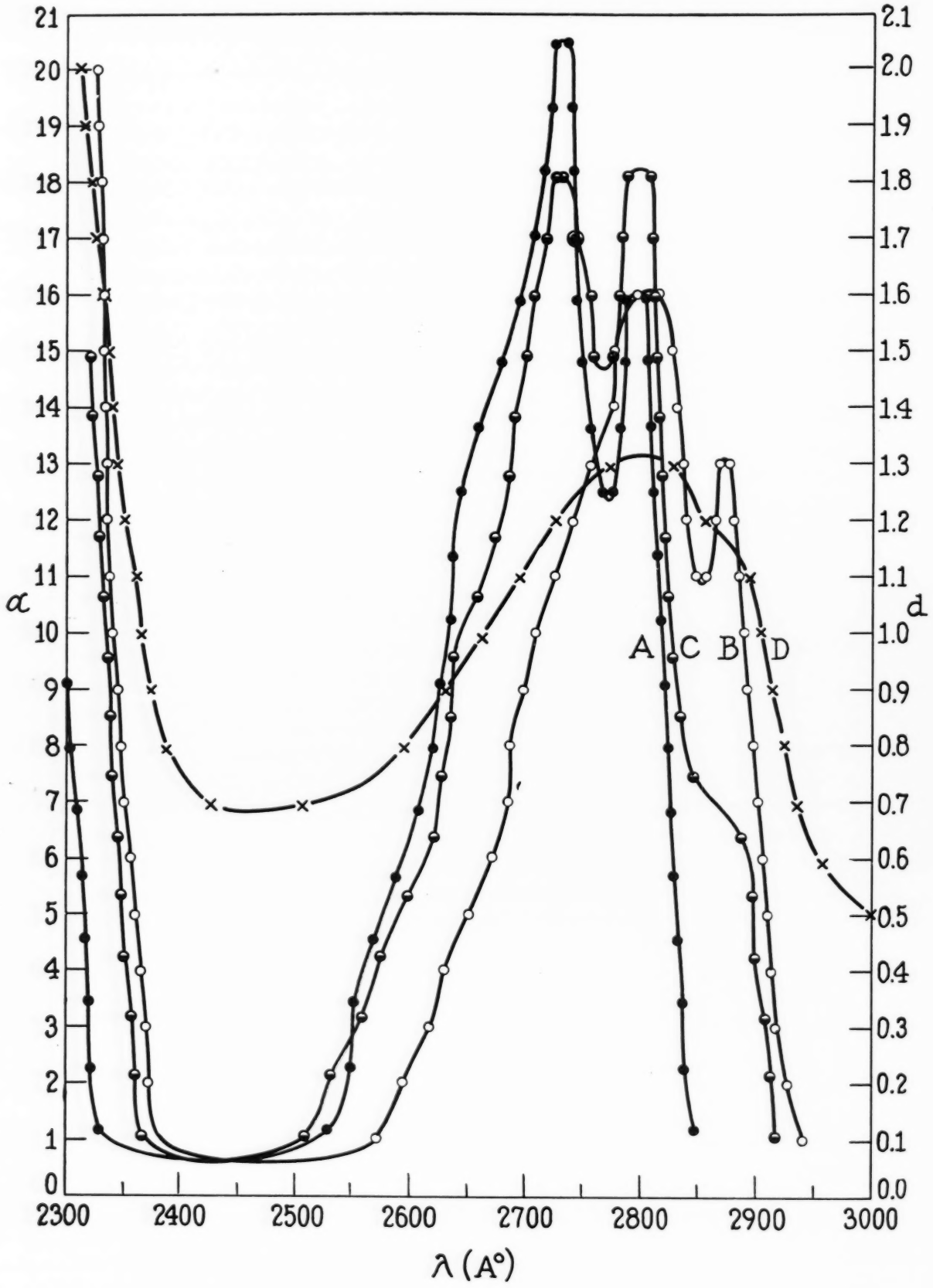


FIG. 17.—Absorption curves of: (A)—Phenol. (B)—*p*-Cresol. (C)—Mixture of phenol and *p*-cresol. (D)—Steam-volatile phenols from hydrolyzed urine (BE7).

lated from urines. These limitations can be partially overcome by more extended fractionation procedures. The differentiation of phenol from *p*-cresol in the same mixture is easily made spectroscopically. Similarly, hydroquinone and catechol, or benzoic acid and phenylacetic acid can be differentiated. Such a differentiation cannot ordinarily be accomplished by color reactions. On the other hand, estrone in a mixture of estrone, estriol, and estradiol can be detected by colorimetric methods. These three female sex hormones give practically identical absorption bands and cannot be distinguished spectroscopically in mixtures. From such data, it follows that spectroscopic and colorimetric methods should be used to supplement each other.

In the various fractions of human urine which have been studied, absorption bands were present which were given by compounds as yet unidentified. Further information concerning these absorbing substances will require their isolation in sufficient quantities to allow chemical study. For their isolation, however, spectroscopic methods will be helpful in following the chemical procedures. On the basis of the findings herein described, it appears that the spectroscopic method is of great value in following the metabolism of certain aromatic substances not normally present in excreta.

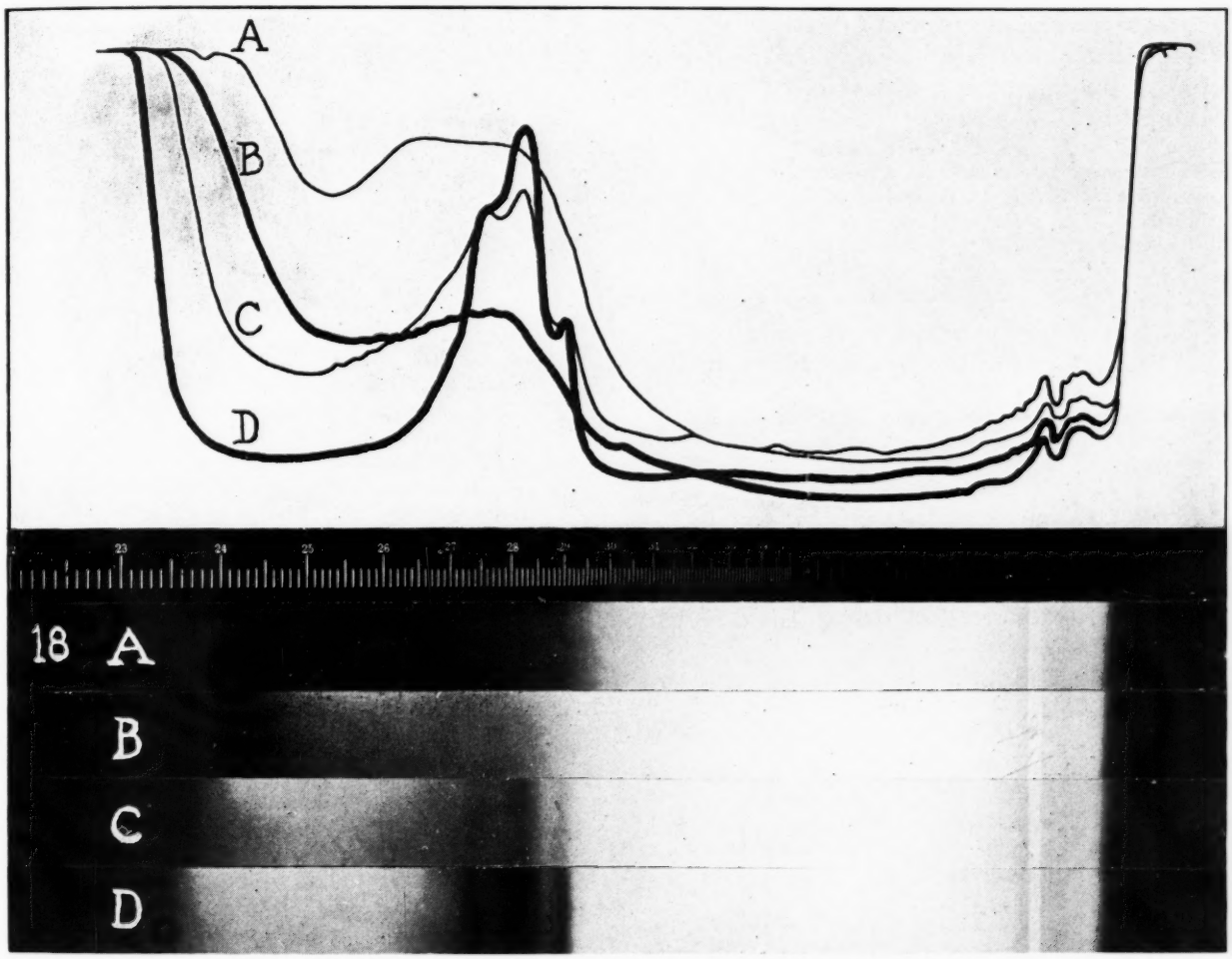


FIG. 18.—Comparison of the band spectra and corresponding photometric tracings. (A)—Ether-soluble phenols from hydrolyzed urine (BE5). (B)—Nonsteam-volatile fraction from (A). (C)—Steam-volatile fraction from (A). (D)—Mixture of pure phenol and *p*-cresol (1 part phenol and 9 parts *p*-cresol).

SUMMARY AND CONCLUSIONS

A method for the treatment and preliminary separation of the ether-soluble substances normally present in urine into groups suitable for the study of absorption spectra is described. Following such a separation, absorption spectra do not present the confused picture of overlapping bands which has been found in the past with whole urine. While no claim is made for complete identification of any one substance, its probable nature may be inferred from comparison with the absorption spectra of certain aromatic substances which are known from previous chemical study to be present in normal urine.

If these pure aromatic substances are mixed and subjected to the fractionation procedures, the resulting absorption bands in the different fractions are in similar positions to the absorption bands from the same fraction obtained from the urine.

Thus far, we have had occasion to study some 150 different urines from 48 normal and diseased individuals, and have not encountered appreciable variations from the findings presented. Possibly the same sort of separation and classification will enable a clearer picture of the absorption spectra of other body fluids to be made.

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The Spectroscopic Study of Biological Extracts

II. The Detection, Isolation, and Biological Effects of the Metabolites of 1,2,5,6-Dibenzanthracene*

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The application of chemical fractionation and qualitative spectroscopic methods to the detection of ether-soluble aromatic compounds from human urine has been reported in a previous publication (14). The results of the spectroscopic survey demonstrated that similar fractions obtained from many individual human urines showed a remarkable uniformity of absorption. A study of the absorptions given by the urine of various animal species (15) indicated the presence of certain differences as compared with the absorptions given by similar fractions from human urines. The bands given by the urine of various individuals of a single species were remarkably uniform. Because of this fact, the occurrence of new bands suggests the presence of compounds not normally present. Therefore, the qualitative spectroscopic method may be employed to follow the metabolism of various compounds which absorb in the ultraviolet region of the spectrum. Many hydrocarbons which possess carcinogenic activity absorb in this region. The fate of anthracene, naphthalene, methylcholanthrene (15), and dibenzanthracene (12) has been investigated by the described combination of chemical fractionation and qualitative spectroscopic methods. This publication is concerned with the metabolic fate of 1,2,5,6-dibenzanthracene [1] in several different animal species.

MATERIAL

Mice, rats, rabbits, dogs, and monkeys were employed. The animals were maintained on normal laboratory diets of constant composition and were kept in standard metabolic cages which were cleaned with alcohol and ether after each collection of the excreta. The urine and feces were collected in 3- or 4-day periods. Adequate control periods were allowed before injection. 1,2,5,6-Dibenzanthracene (DBA) was injected subcutaneously employing a solution of 500 mgm. of the white, colorless compound (Eastman) dissolved in 75 cc. of olive oil. Animals of the various species received the following doses of DBA once or twice weekly: mice, 10 to 20 mgm.; rats, 20 to 30 mgm.; rabbits, 50 mgm.; monkeys, 200 mgm.; dogs, 500 mgm. For the spectroscopic survey, the excreta were worked up at the end of each 3-day period. For the isolation procedures, the excreta from several collection periods were pooled and were kept in the refrigerator.

METHODS

I. Chemical

The methods for the extraction and fractionation of the ether-soluble compounds from the excreta and tissues were, with some minor modifications, the same as those described in a previous publication (14).

a. *Urine and bile.*—The ether-soluble fractions from urine and bile were prepared by a continuous ether extraction for 72 hours in a Kutcher-Steudel apparatus. Four primary ether extracts were obtained from the same urine or bile treated successively as follows (Fig. 1): 1. First the material was acidified to pH 1 with concentrated hydrochloric acid (ether extract AE1). 2. It was then made alkaline to pH 8 with solid sodium bicarbonate (ether extract BE1). 3. It was reacidified to pH 1 with concentrated hydrochloric acid and refluxed on a hot plate for one-half hour or autoclaved for two hours at 115° C. at 15 pounds (ether extract CE1). 4. The hydrolyzed material was made alkaline with solid sodium bicarbonate to pH 8 (ether extract DE1). The fractions containing the acidic, phenolic, and neutral substances were prepared from the primary ether extracts obtained from both nonhydrolyzed (AE1) and hydrolyzed urine or bile (CE1). The primary ether extracts, BE1 and DE1, contained the basic compounds obtained from the nonhydrolyzed and hydrolyzed urines respectively. The ethereal subfractions were brought to a convenient

* This investigation was aided by grants from the Rockefeller Foundation and from The Jane Coffin Childs Memorial Fund for Medical Research.

[1] Henceforth, "DBA" will be used in place of "dibenzanthracene" except in special instances.

volume (10 to 50 cc.) on the steam bath and their absorption spectra in the ultraviolet region were taken.

b. *Feces and intestinal tract content.*—The feces were acidified with concentrated hydrochloric acid, mixed with anhydrous sodium sulfate, ground to a fine powder, and mixed with sand. They were then extracted with ether for 72 hours in a Soxhlet apparatus. The ether extracts were fractionated as described for extracts of urine (see section a.),

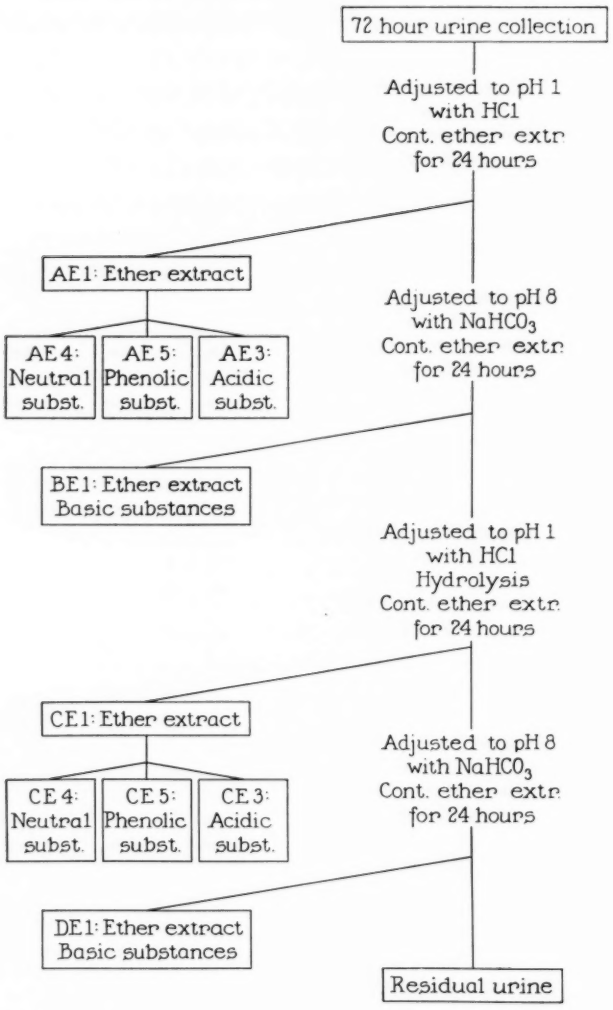

FIG. 1.—Scheme for the separation of the ether-soluble substances from urine, feces, and tissues.

brought to a volume of 20 to 100 cc., and examined spectroscopically.

c. *Tissues.*—The tissues were ground up in a mortar and extracted thoroughly with a mixture of alcohol and ether. The extracts were fractionated as described under a.

d. *Whole rats.*—Carcasses of DBA-injected rats were ground up and saponified with 10 per cent alcoholic potassium hydroxide. After acidification with hydrochloric acid, the material was extracted for 72 hours with ether. Fractionation of the ether extract was carried out as described under a.

II. Spectroscopic

The spectroscopic methods used were described in a previous publication (14).

RESULTS

I. Spectroscopic Survey of the Crude, Neutral, Phenolic, Acidic, and Basic Fractions

For this survey, the absorption spectra of similar fractions obtained from the excreta and tissues of animals before and after the injection of DBA were compared. By this procedure, additional absorption bands which were present after the injection of DBA could be detected. The bands present in the fractions of the material from normal animals will not be discussed in detail if not relevant to the detection of new absorption bands which could be attributed to the presence of DBA or its derivatives. The results obtained are summarized in Table I.

A. *Fraction containing the neutral, ether-soluble compounds.* 1. *Urine.*—In this fraction (AE_4) of the urine from some uninjected animals, particularly monkeys, bands in positions identical with those given by indigo red were observed. After the injection of DBA, this fraction from the urine of rabbits and monkeys occasionally gave a series of sharp bands which were not present in the similar fractions from the urine of normal animals. Similar bands were usually present in the neutral fraction from the urines of DBA-injected rats and mice. After the hydrolysis of the urines, these bands could not be found. The positions of the bands were identical [2] with those given by an ethereal solution of DBA. Those given by the urinary fractions were of comparatively low intensity and were obscured and diminished by a relatively large amount of background absorption. The spectroscopic findings suggested the presence of DBA in the urine of the animals injected with that compound.

The possibility of a leakage after injection of the olive oil containing DBA cannot be excluded. Furthermore, the urine may wash down fecal particles. The spectroscopic suggestion of the presence of DBA in the urines of injected rabbits and monkeys may result from this artifact, because the bands are not found regularly. In the case of injected rats and mice, on the other hand, the regular occurrence of these bands indicates that these species may excrete a small amount of DBA in the urine.

2. *Feces.*—The neutral fraction (AE_4) from the

[2] Hereafter the term "identical" as applied to absorption bands will be used to designate bands in identical positions.

feces of normal animals was highly pigmented and contained a relatively large amount of background absorption. Occasionally diffuse absorption bands of relatively low intensity were present. In the fraction obtained from the feces of mice and rats after DBA injection, bands in positions identical with those given by DBA were regularly present. Their intensity in the region of 3,100 to 4,500 A. was often considerably decreased due to background absorption. In the fraction obtained from feces of injected rabbits, dogs, and monkeys, bands of low intensity in positions identical with those of DBA were observed occasionally, usually in the first collection after injection. In the neutral fecal fraction from certain uninjected monkeys, a series of diffuse bands between 3,650 A. and 3,900 A. and four well-defined bands in the region of 3,000 A. to 3,500 A. are occasionally present, but if present, the bands of DBA could readily be detected and distinguished.

4. *Bile.*—Only a few cubic centimeters of bile could be obtained from rabbits injected with DBA. After fractionation of the material, the neutral fraction showed only faint bands suggesting the presence of the hydrocarbon.

5. *Livers.*—The neutral fractions of mouse, rat, and rabbit livers of animals injected with DBA showed spectroscopically a number of bands different from those of DBA. A band at 2,990 A., seen in the neutral fraction obtained from the rats and rabbits, might indicate the presence of a trace of DBA. In the same fraction obtained from mouse livers, there was no suggestion of a band in this position.

6. *Whole rats.*—The neutral fraction from rats injected with DBA showed intense bands identical with those of DBA.

B. *Fractions containing the phenolic compounds.* 1. *Urine and feces of rats and mice.*—In this fraction obtained from the urine of uninjected animals (AE5),

TABLE I: SUMMARY OF THE EXCRETION AND CONVERSION OF 1,2,5,6-DIBENZANTHRACENE (DBA) AND DIHYDROXYDIBENZANTHRACENE IN DIFFERENT SPECIES

	DBA Neutral fraction					$(OH)_2$—DBA Phenolic fraction				
Animals	Urine	Feces	Intestinal tract contents	Liver	Bile	Urine	Feces	Intestinal tract contents	Liver	Bile
Mice	+	+	(+)	o	..	+	+	(+)?	o	
Rats	+	+	+	(+)	..	+	+	+	o	
Rabbits	(+)?	(+)	(+)	(+)	(+)	+	+	+	o	(+)
Dogs	(+)?	(+)?	..	..	..	(+)	(+)	..	..	
Monkeys	+	o	..	o	..	.		..	..	

+ Positive; (+) traces; (+)? questionable; o negative; .. not studied.

The excretion of DBA in the feces of rats and mice is suggested by the regular finding of absorption bands in positions identical with those of DBA. In rabbits, dogs, and monkeys, however, this finding appears to be due to the leakage of oil containing DBA.

3. *Intestinal contents.*—It was uncertain whether the DBA detected spectroscopically in the fractions from the urine and feces of certain species represented an excretory product or whether its presence in these fractions was an artifact. Therefore, experiments were undertaken to elucidate this point.

In the neutral fraction obtained from the intestinal contents of mice injected with DBA, bands of very low intensity in positions identical with DBA were present. The similar fraction from the intestinal contents of rats showed the same bands in greater intensity. In the same fraction of rabbits, the bands of DBA were present in low intensity.

From this experiment it appears that the presence of DBA in the feces of mice, rats, and rabbits is not due to an artifact, but is the result of the excretion of DBA into the intestinal tract following parenteral injection.

diffuse absorption bands were found in the region of 2,600 A. and 2,900 A. In similar fractions obtained from the urines after DBA injection, groups of bands were observed which were not present in this fraction from the urine of normal animals. The bands were in the following positions: a strong band at 2,880 A., 3 bands of low intensity between 3,200 A. and 3,500 A., and 2 sharp bands between 3,800 A. and 4,100 A. The absorption bands in the fractions from mice and from rat urines were identical. The spacing of the bands was similar to that of DBA, but a comparison of the exact positions of the bands with those of DBA shows that the former are shifted by about 50 A. towards the higher wave lengths. The absorption spectra given by similar fractions of hydrolyzed urine or normal and DBA-injected animals showed no bands suggesting the presence of DBA metabolites.

In the phenolic fraction obtained from the feces of normal animals, a material was present which gave a diffuse absorption from 2,700 A. to 2,900 A. and from 3,000 A. to 2,500 A. The intensity of the bands varied in different preparations. In the fractions of the feces of the DBA-injected animals, bands were

observed in positions identical to those described for the urine of the injected animals.

In the phenolic fraction of the urine and feces from rats and mice injected with DBA, a series of bands was observed which are similar in grouping to, but in different positions from, the bands characteristic of DBA, a fact which indicated the presence of a phenolic derivative of DBA. The same metabolite may be produced by rats and mice, since the same bands are observed in the phenolic fractions from these two species.

2. *Urine and feces of rabbits.*—In the phenolic fraction of the urines of uninjected rabbits, diffuse absorption bands were present somewhat similar to those found in the same fraction of the urine from mice and rats. Of a series of sharp bands given by the fraction obtained from the urines of animals injected with DBA, two were relatively intense in the region of 2,850 A. to 3,050 A.; three bands of lower intensity were present in the region of 3,800 A. to 4,100 A. A comparison of the absorptions given by the fraction obtained from the urines after hydrolysis showed no difference between the excreta of normal and of DBA-injected animals.

A varying amount of background absorption was given by the phenolic fractions of the feces obtained from normal rabbits. In the similar extract of the feces of animals injected with DBA, bands were present in positions identical with those given by the same urinary fraction.

The bands observed in the phenolic fraction from the urine and feces of injected rabbits showed groupings somewhat similar to those of DBA but slightly shifted towards the longer wave lengths. A comparison of the positions of the absorption bands given by the phenolic fraction of the excreta of injected rabbits with the absorption bands given by the DBA derivatives excreted by injected rats and mice showed that the shift of the bands of the rabbit compound in the region of 3,000 A. was less than that of the compound excreted by rats and mice; in the region of 3,100 A. to 3,500 A., the shift of the bands of the rabbit compound was greater, and the two bands in the region 3,800 A. to 4,110 A. were in identical positions.

The presence of bands in the phenolic fraction of urine and feces of injected rabbits in positions similar to, but not identical with, those of DBA suggests the presence of a phenolic derivative of DBA. The comparison of the positions of the bands given by the phenolic fraction of rabbits' excreta with those given by a similar fraction of the excreta of rats and mice shows that the positions are not identical. This difference of position indicates that the phenolic derivative of DBA excreted by rabbits is a different compound from that excreted by mice and rats. The lack of similar bands in the urines after hydrolysis indicates that the phenolic compound is not freed by the hydrolysis of an ether-soluble ethereal sulfate or glucuronide.

3. *Urine and feces of monkeys and dogs.*[3]—The phenolic fractions of urines and feces of monkeys both before and after hydrolysis and before and after the injection of DBA showed no differences in ultraviolet absorptions; no bands were observed which were suggestive of a phenolic derivative of DBA in these two species.

The phenolic fractions of the urines and feces of dogs injected with DBA showed faint absorption bands in the region of 2,900 A. to 3,100 A. which were not present in the same fractions obtained from the excreta of uninjected dogs.

4. *Intestinal tract contents.*—The phenolic fraction obtained from the intestinal contents of mice injected with DBA showed no bands characteristic of a DBA derivative. In the phenolic fraction of rats injected with DBA, bands of a relatively high intensity were present and the positions of these bands were identical with those of the excreted phenolic conversion product found in rat urine and feces. The phenolic fraction from DBA-injected rabbits showed a series of bands of high intensity whose positions corresponded with those of the conversion product present in the urine and feces of rabbits.

5. *Bile.*—In the phenolic fraction from rabbit bile, a number of bands different in position from the phenolic conversion product excreted in urine and feces were observed. One sample, however, showed bands in positions identical with those of the excreted phenolic conversion product.

6. *Livers.*—In the phenolic fractions obtained from the livers of mice, rats, and rabbits injected with DBA, no bands were observed which could suggest the presence of a phenolic conversion product of DBA.

7. *Whole rats.*—In the phenolic fraction obtained from whole rats injected with DBA, a large amount of generally absorbing material obscured faint bands in positions identical with the phenolic conversion product of DBA found in the urine and feces of injected rats.

C. *Fractions containing the acidic compounds, AE3 and BE3.*—A comparison of the bands given by this fraction obtained from the urine and feces of animals of all species investigated before and after the injection of DBA showed no bands which suggested the presence of a DBA derivative.

[3] Since this manuscript was submitted, the phenolic fraction of the urine of dogs injected with DBA was purified further. A phenolic DBA derivative is present which differs spectroscopically both from the phenolic derivatives excreted by rats and mice, and by rabbits.

D. *Fractions containing the basic compounds.*—The basic fraction of urines of mice, rats, rabbits, and dogs gave no bands which suggested a basic derivative of DBA. The similar fraction from the urine of injected monkeys showed irregularly a series of bands which were not present in the basic fraction of normal monkeys. Studies on the nature of this absorbing material are in progress.

E. *Investigation of the presence of ether-insoluble DBA derivatives.*—The presence of ether-insoluble derivatives of DBA was investigated in the urines of different species after hydrolysis and autoclaving of the urine. By this treatment ether-soluble DBA or DBA derivatives may be split off from ether-insoluble conjugated DBA compounds. In the phenolic fraction from rabbit urines, bands of low intensity were only very rarely observed in positions identical with the bands of the phenolic derivative obtained from unhydrolyzed urine. The fractions obtained from the urines of other species showed no bands which indicated the presence of DBA or a DBA derivative.

The unhydrolyzed urines of rabbits and rats, after the preliminary ether extraction, were extracted with butyl alcohol or chloroform solvents which may dissolve conjugated DBA derivatives. After these fractions had been taken up in alcohol and examined spectroscopically, no suggestion of a DBA derivative was found.

In the course of the fractionation procedure DBA derivatives might be rendered less ether-soluble. Therefore, precipitates which formed during the fractionation procedure were dissolved in alcohol and absorption spectra were taken. However, no suggestion of a DBA derivative was found.

II. The Purification and Isolation of the Phenolic Derivatives of DBA from the Excreta of Mice, Rats, and Rabbits (12)

The phenolic derivatives of DBA excreted by mice and by rats were isolated from the combined urine and feces of each species. In rabbits, the phenolic derivative was isolated both from the urine and feces. The excreta were collected from 50 mice, 30 rats, and 16 rabbits. The urine and feces were kept in the icebox and worked up in pooled collections of the material excreted during 10 days. The ether-soluble phenolic fractions were obtained as described under METHODS, and the fractions from each 10-day period were combined for the final purification procedures. With minor variations, the same technic was used for the excreta of all the species employed. All purification procedures were controlled by fluorescence and spectroscopic methods.

A. *Primary purification of the phenolic fraction.*—The combined ethereal solutions containing the phenolic compounds were extracted with 10 per cent sodium carbonate until no further pigments could be removed. The ethereal solution was then extracted several times with 10 per cent sodium hydroxide. The combined sodium hydroxide solutions were acidified with hydrochloric acid and extracted three times with ether. The procedure was repeated several times. By this means, relatively large amounts of impurities were eliminated with a minimal loss of the phenolic compounds as judged by the spectroscopic method. The ether was evaporated and the oily residue was dried in a vacuum desiccator and submitted to high vacuum distillation as described below.

B. *High vacuum distillation.*—High vacuum distillation was carried out on a Hickman oil diffusion pump at a pressure between 10^{-4} and 10^{-5} mm. of mercury. Preliminary experiments showed that fractions collected between the following temperatures were satisfactory: 25-50° C., 50-90° C., 90-150° C., 150-250° C. In the fraction collected between 25-50° C., a considerable amount of oily material, phenol, and *p*-cresol were present. This fraction was discarded since it gave no bands spectroscopically which indicated the presence of a DBA derivative. In the fraction collected between 90 and 150° C., only small amounts of DBA derivatives were detected spectroscopically. In the fraction collected between 150 and 250° C., most of the derivative was present. Although no quantitative studies have been made, it appears that at high temperatures some destruction of the DBA derivatives takes place.

C. *Chromatographic adsorption analysis.*—Since the fractional crystallization of the distillates collected between 90 and 150° C. and 150 and 250° C. was not successful, except for the material from the urinary fraction of injected rabbits, further purification was carried out by chromatographic adsorption analysis. Preliminary studies controlled by spectroscopic and fluorescence procedures showed that the following technic was satisfactory: Aluminum oxide Brockmann (Merck) was used as the adsorbent. The bore of the adsorption columns varied from 10 to 20 mm. in diameter. The amount of aluminum oxide used was roughly 50 times the weight of the material to be purified. The height of the adsorption column was 20 to 30 times that of the diameter of the glass column used. The solvents were forced through the column by air pressure supplied by water flowing from a height of 5 feet into a closed container. A calcium chloride tube was inserted between the closed container and the column to absorb the water vapors. The columns were prepared in a mixture of benzol and ether (1:1), and the following solvents were used successively in 100 cc. portions: ether-benzol (1:1), ether, ether-acetone (9:1), ether-acetone (1:1),

acetone, acetone-methyl alcohol (9:1), acetone-methyl alcohol (1:1), methyl alcohol, ethyl alcohol, and ethyl alcohol-water (1:1). Since DBA and DBA derivatives give strong fluorescence, the quantity of a solvent necessary for the elution of the fluorescent materials from the column was controlled by means of a mercury lamp with a Wood filter. The rabbit and rat derivatives were eluted in mixtures of acetone and methyl alcohol. The use of the complete sequence of solvents described is of value in that a large amount of oil and pigments may be removed before the elution of the desired material. Because of the differences in the impurities which influence the speed of elution in each preparation, the exact point in the sequence of solvents at which the DBA derivative will be eluted cannot always be predicted. The different eluates were evaporated *in vacuo,* dissolved in measured amounts of ether, and investigated spectroscopically. The fractions containing the DBA derivative were combined and the chromatographic procedures were repeated two or more times, according to the amount of impurities present. The compound excreted by rabbits is eluted from the column earlier in the sequence of solvents than is the rat compound. This fact is in agreement with the higher solubility in ether of the pure compound produced by rabbits than of the rat compound. The details of a typical chromatographic adsorption analysis are shown in Table II.

D. *Isolation and properties of the phenolic derivative.*[3]—Preparations from the same species which gave fluorescence and strong ultraviolet absorption were combined, purified in alcoholic solution with a very small amount of Norite, and crystallized from mixtures of acetone and petroleum ether. The melting points of the different preparations are given in Table III. The material isolated from the urine of rabbits melted at 355-358° C. The elementary analyses were: Calcd. for $C_{22}H_{14}O_2$: C, 85.14; H, 4.54. Found: C, 85.04; H, 5.07.

The mixed melting point of equal amounts of the rabbit compound with the rat compound gave a melting point of 335-339° C., a depression of 20° C. A melting point in a closed tube of the rat compound as determined by Fieser and Cason was 390-400° C. corr. (7). They reported that this preparation did not give any depression of the mixed melting point with the synthetic 4′,8′-dihydroxydibenzanthracene. The synthetic compound of Fieser and Cason (7) melted at 415-418° C. corr.

TABLE II: CHROMATOGRAPHIC ANALYSIS OF CRUDE DIHYDROXYDIBENZANTHRACENE PREPARATIONS

Solvent cc.*		Rabbits		Rats	
		Fluorescence	Spectroscopic	Fluorescence	Spectroscopic
EB 1:1	50	Blue	....	Pale blue	....
EB 1:1	50	Blue	....	Blue	....
E	50	Pale blue	....	Violet	....
E	50	....	....	Light blue	....
10% AcE	50	....	....	Blue +	....
50% AcE	50	....	....	Blue +	....
Ac	50	Light green	Phenol *p*-cresol	Blue +	....
Ac	50	Blue +++	(+)	Blue +	....
10% MA Ac	50	Blue +++	++	Blue +	....
10% MA Ac	50	Blue +++	+++	Blue +	....
10% MA Ac	50	Blue +++	+++	Blue +(+)	(+)
50% MA Ac	50	Blue +++	+++	Blue +++	+++
50% MA Ac	50	Blue +++	++	Blue +++	+++
MA	50	Blue ++	+	Blue +++	+
EA	50	Blue +	(+)	Blue +	(+)

* E = ether; B = benzol; Ac = acetone; MA = methyl alcohol; EA = ethyl alcohol.

TABLE III: MELTING POINTS OF NATURAL AND SYNTHETIC DIHYDROXYDIBENZANTHRACENES

Animals	Source	Dobriner *et al.*	Cason and Fieser (7)	Cason and Fieser (7)	Boyland and Levi (5)
Rabbits	Urine	355-358° C.	.....	.....	340-350° C.
Rabbits	Feces	356° C.—a‡	.....	.....	.....
Rats	Urine and feces	Above 370° C.—b‡	390-400° C.*	415-418° C.†	.....
Mice	Urine and feces	Above 370° C.	.....	.....	.....

* Melting point of the natural material isolated by Dobriner *et al.*
† Melting point of synthetic 4′,8′-dihydroxydibenzanthracene.
‡ Mixed melting point of a and b, 335-339° C.

[3] The authors wish to thank Dr. L. C. Craig of the Rockefeller Institute for Medical Research for the purification and recrystallization of the isolated dihydroxydibenzanthracenes.

III. Spectroscopic Investigation of the Purified Compounds

The absorption spectra of DBA and the phenolic derivatives of rabbits, rats, and mice are shown in Fig. 2. The absorption curves of the phenolic derivatives from rats and mice are identical. The spectra were measured by means of a small Hilger spectrograph, a Spekker photometer, and a metallic spark as a light source. All the substances measured were dissolved in absolute ethyl alcohol.

In Fig. 3 are shown the absorption bands of the same compounds as measured by the Hilger spectrograph with a hydrogen discharge tube as a source of light. The two lower spectra (II and III) are those given by dilutions of the solutions from which the top spectra (I) were obtained. Those marked "A" represent the bands given by DBA, those marked "E" represent the ones given by the phenolic derivative from rabbits, "C" indicates the bands given by the phenolic derivative of rats, and "D" those from mice.

In the same figure the positions of the phenolic derivative from rats and mice is compared with the bands (B) given by the synthetic 4′,8′-dihydroxydibenzanthracene obtained through the courtesy of Dr. Fieser. The absorption spectra were taken in alcoholic solutions. The positions of the bands from the natural DBA derivative excreted by mice and rats and the synthetic product are identical.

In Fig. 4 the absorption curves given by the phenolic derivative of rabbits isolated in this laboratory (b) and by the phenolic derivative of DBA isolated from rabbit urine by Boyland and Levi (5) are depicted (a). The shapes of the curves are identical.

IV. Tests for Carcinogenic Activity of the Rabbit Compound

Twenty-two mice of a pure strain of known susceptibility to DBA were obtained through the courtesy of Dr. Clara Lynch of the Rockefeller Institute for Medical Research. Eleven animals were employed to test the carcinogenicity of the phenolic derivative of DBA. Each animal was injected with 1 mgm. on the following dates: 11/28/39 and 12/2/39, and with 2 mgm. on 1/4/40 with DBA or the phenolic derivative of DBA, respectively. The solution injected contained 4 mgm. of DBA, or the phenolic derivative, per cc. of lard. Two of the animals injected with the phenolic derivative died after 3 weeks without evidence of a tumor. The 11 mice injected with DBA and 9 remaining mice injected with the phenolic derivative were killed 240 days following the first injection. All the DBA-injected animals showed at autopsy a sarcoma at the site of injection, and none of the 9 mice injected with the phenolic derivatives of DBA developed tumors.

V. Fate of the Dihydroxydibenzanthracene Excreted by Rabbits after Injection in Mice

The urine and feces of the mice injected with the phenolic derivative of DBA excreted by rabbits were collected for 6 days following the first injection and fractionated as described under METHODS. The absorption spectra of the neutral and phenolic fraction were investigated. In the neutral fractions of the urine and feces, no bands were seen which were characteristic of DBA or of a DBA derivative. In the phenolic fractions from urine and feces, respectively, bands were present in positions identical with the bands given by the phenolic derivative excreted by rabbits. In the urine, only traces of this material were present, while in the feces, a relatively high concentration was observed, as judged by the results of the spectroscopic method.

VI. The Comparative Ability of Different Species to Convert DBA

It has been demonstrated that different species show varying susceptibility in tumor development due to the administration of DBA (6). Furthermore, this group (12) and Boyland and Levi (5) have presented evidence that the phenolic metabolite of DBA excreted by rabbits does not produce tumors in mice. It was of interest, therefore, to investigate in various species the relative speed of conversion of DBA, as well as the rate of excretion of DBA and its conversion products.

Twenty mice, 6 rats, and 2 rabbits were injected with DBA dissolved in olive oil. Four hundred mgm. were administered to each group of animals. The urine and feces were collected for a 6-day period. The animals were killed; the total urines, feces, and intestinal tract contents of the animals in each species were worked up as described under METHODS. The experiment was repeated (Table IV).

Table IV: Excretion and Conversion of 1,2,5,6-Dibenzanthracene (DBA) in Groups of Animals of Different Species

Animals *		DBA mgm.				Dihydroxy DBA			
		Urine	Feces	Intestine	Total	Urine	Feces	Intestine	Total
20 Mice	a	..	..	..	..	0.08	1.2	..	1.3
	b	0.3	5.0	0.002	5.3	0.06	0.6	..	0.7
6 Rats	a	0.4	3.3	..	3.7	..	..	..	..
	b	0.4	3.6	1.2	5.2	0.2	0.8	0.2	1.2
2 Rabbits	a	..	0.7	..	0.7	4.0	2.0	..	6.0
	b	..	..	0.7	0.7	16.5	0.4	0.7	17.6

* 400 mgm. DBA injected in each group of animals. Collection period of 6 days.

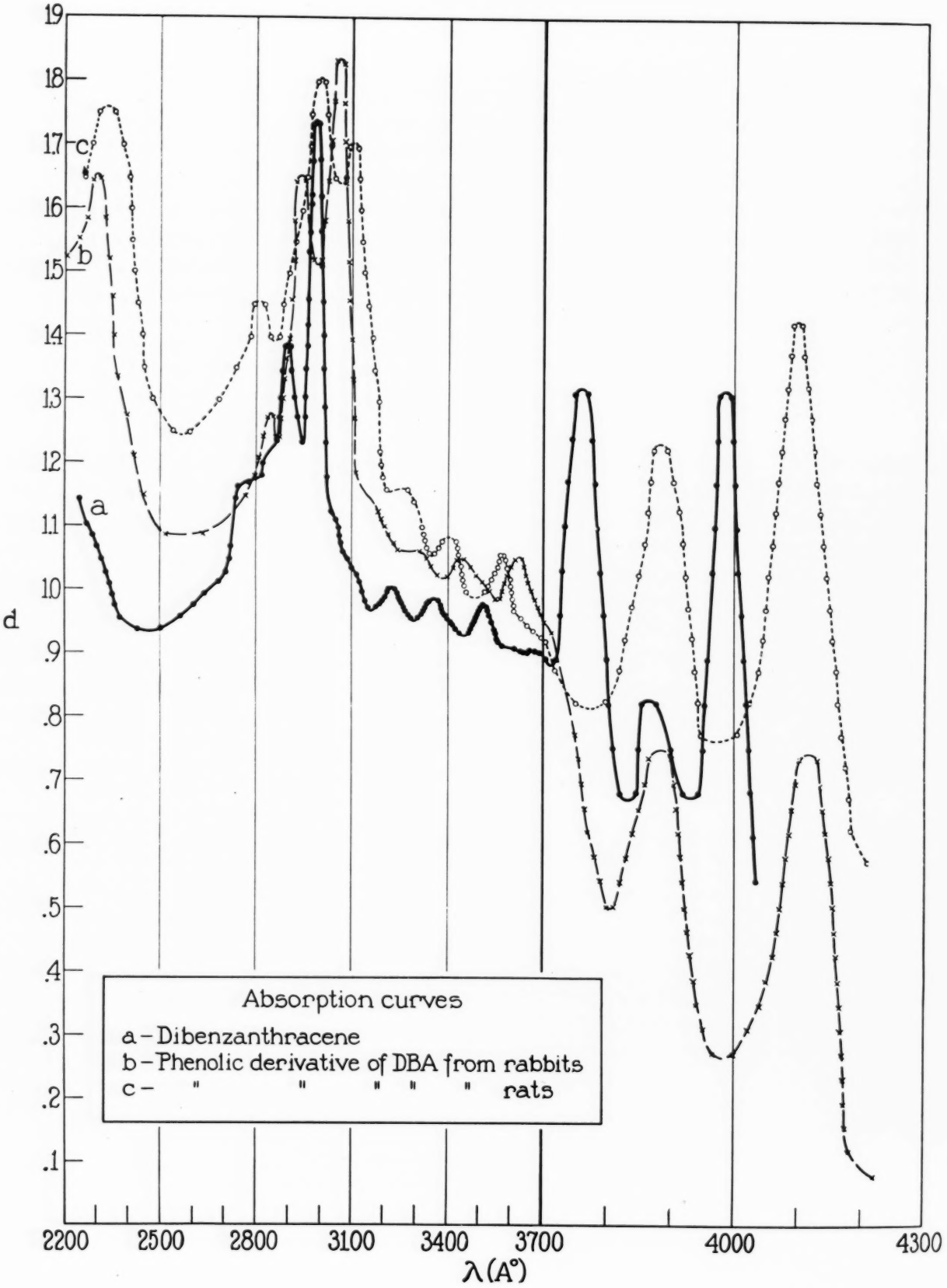
19
18
17
16
15
14
13
12
11
10
.9
.8
.7
.6
.5
.4
.3
.2
.1
d
c
b
a
Absorption curves
a – Dibenzanthracene
b – Phenolic derivative of DBA from rabbits
c – " " " " " rats
2200
2500
2800
3100
3400
3700
4000
4300
λ (A°)

FIG. 2

A rough quantitative estimation of DBA present in the neutral fractions and of the phenolic conversion products present in the phenolic fractions was made. The sharp band of DBA present at 2,990 A. was diluted out. The series of dilutions were compared with similar dilution of standard solutions of pure DBA and of the phenolic conversion products. Since the neutral as well as the phenolic fractions from the three species contained a variable amount of background material which decreases the intensity of the bands, the quantitative estimation is very crude. Nevertheless, if large differences in the conversion and excretion rates are present, an indication may be obtained with regard to species differences.

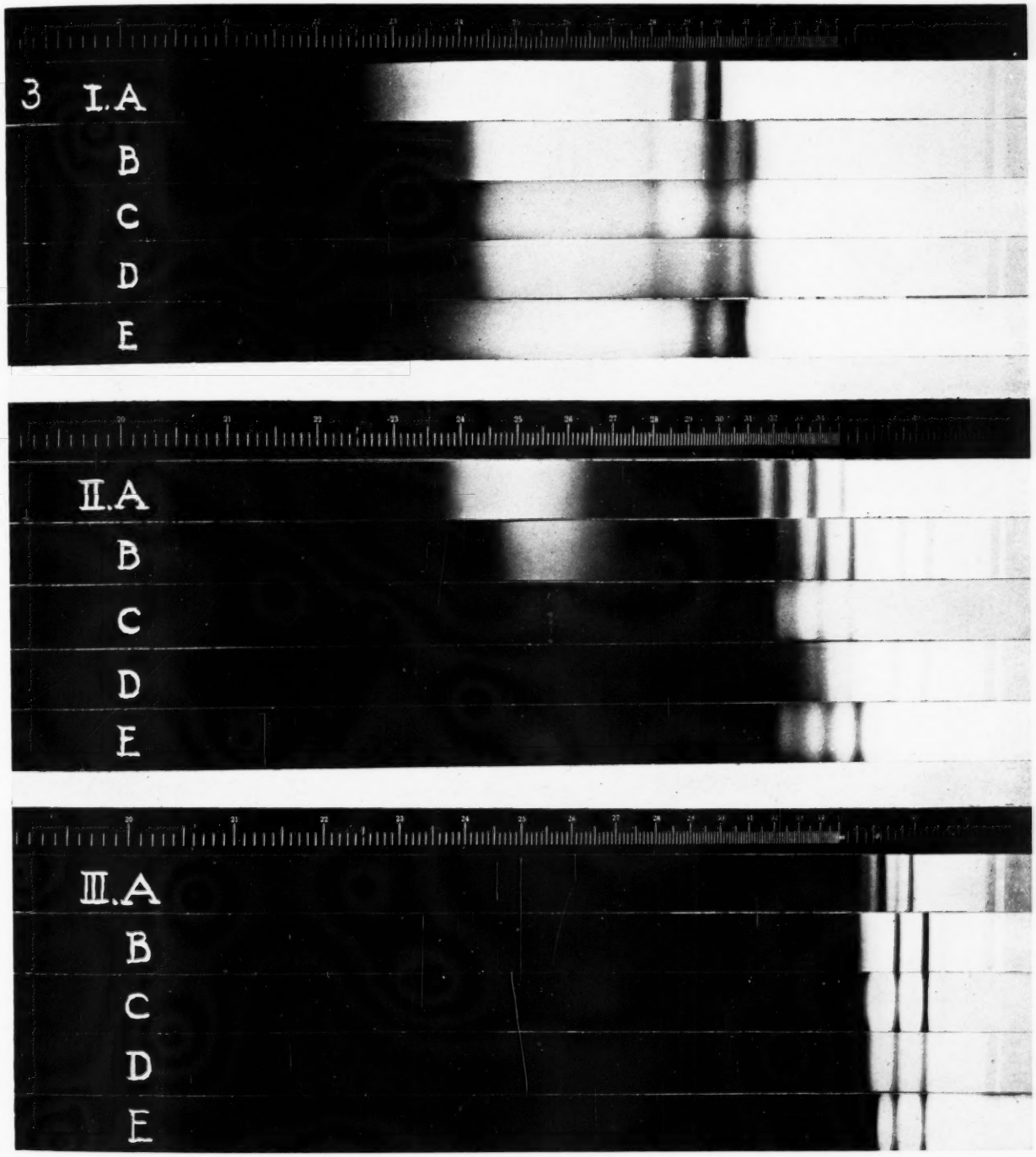

FIG. 3.—Comparison of the absorption bands given by: (A)—1,2,5,6-Dibenzanthracene. (B)—4′,8′-Dihydroxydibenzanthracene synthesized by Fieser and Cason. (C)—Dihydroxydibenzanthracene excreted by rats after the injection of 1,2,5,6-dibenzanthracene. (D)—Dihydroxydibenzanthracene excreted by mice after the injection of 1,2,5,6-dibenzanthracene. (E)—Dihydroxydibenzanthracene excreted by rabbits after the injection of 1,2,5,6-dibenzanthracene.

As seen in Table IV, mice and rats excrete a relatively large amount of DBA as compared to rabbits. Mice and rats convert only a small amount of the injected DBA to a phenolic derivative as compared to rabbits. Moreover, the excretion and conversion of DBA is extraordinarily low in all species. These results are preliminary.

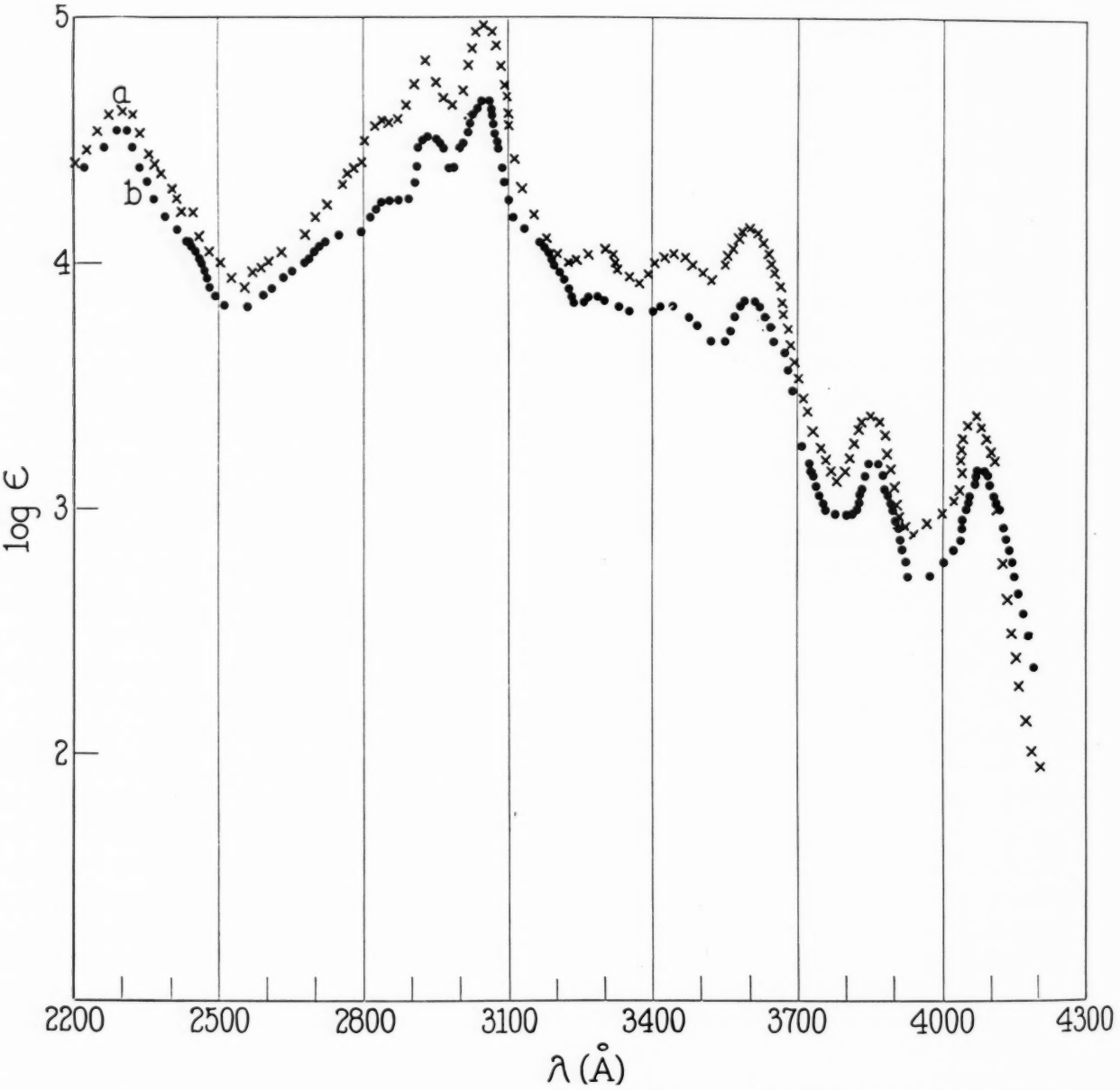

FIG. 4.—Absorption curves of dihydroxydibenzanthracene: (a)—Isolated by Boyland and Levi (5). (b)—Isolated by Dobriner, Rhoads, and Lavin.

DISCUSSION

The value of the application of the spectroscopic method to the study of the metabolism of DBA is demonstrated by the following observations on the excreta of the DBA-injected animals: 1. In the crude ether extracts containing neutral substances, a series of bands identical in position with those of DBA was observed. 2. In the crude ether extracts containing the phenolic substances, a series of bands similar to but not in identical positions with the bands of DBA was seen. These bands suggest a phenolic conversion product of DBA. 3. The series of bands in the phenolic fraction obtained from the excreta of rats and of mice differed in their positions from those of the bands obtained from the same fraction of rabbit urine and feces.[3] 4. The monkeys provided no evidence of excretion of DBA or an ether-soluble phenolic metabolite of DBA. These observations on the crude fractions suggest that rats and mice excrete the same phenolic DBA derivative, but that

this derivative is not identical with the phenolic conversion product excreted by rabbits. The sensitivity of this qualitative method is demonstrated by these observations inasmuch as the amount of DBA and its metabolites present in 72-hour urine and feces collections is extremely small (Fig. 4).

As pointed out in the first publication, there are factors which decrease the sensitivity of the spectroscopic methods. One of the most disturbing is the varying amount of background absorption which is given by all the fractions obtained from natural material. This background absorption renders invisible bands given by substances present in lower concentration. Thus, all the statements which concern negative findings must be interpreted as indicating that under the spectroscopic conditions applied no bands could be detected. This applies particularly to the study of tissue extracts. Further purification of the extracts by improved methods may provide evidence of the presence of traces of materials which give absorption in the ultraviolet region of the spectrum.

In the course of the isolation procedures, the spectroscopic method was extremely useful in its application to the fractions obtained by high vacuum distillation and chromatographic adsorption analysis. From the information thus obtained, it was possible to discard large amounts of residual material and to concentrate the small amounts of DBA metabolite present. The qualitative and quantitative spectroscopic analyses of the isolated compounds are shown in Figs. 3 and 2. Both spectroscopic methods show the identity of the absorption of the phenolic derivative obtained from rats and from mice, and the difference as compared with the phenolic derivative obtained from rabbits. The positions of the bands of the rabbit compound are identical with those of the dihydroxydibenzanthracene isolated by Boyland and Levi (Fig. 4).

The phenolic derivatives from the urine and feces of rabbits, rats, and mice were isolated from the phenolic ether-soluble fraction of the excreta by means of high vacuum distillation, chromatographic adsorption analysis, and recrystallization. Evidence from the melting points and mixed melting points of the phenolic derivatives of DBA demonstrate that rats and mice excrete a different compound than do rabbits. These facts agree with the evidence derived by the spectroscopic methods. Boyland and Levi (4, 5, 21) isolated dihydroxydibenzanthracene from the urines of rabbits fed with DBA. The chemical and physical properties of the compound isolated by us are the same as those of the compound isolated by Boyland and Levi. The positions of the hydroxyl groups in the compound excreted by rabbits have not yet been established. The problem is under investigation by Boyland and Levi (5) and by Cason and Fieser (8).

Fieser and Cason (7) synthesized 4′,8′-dihydroxy-1,2,5,6-dibenzanthracene. From their evidence on melting points and mixed melting points, the synthetic compound is identical with the phenolic compound isolated from the excreta of rats. The absorption spectrum of the synthetic compound as determined by Jones and Fieser, and by our group, is identical with the absorption of the DBA metabolite excreted by rats.

The possibility of the presence in the urine of conjugated derivatives of DBA and hydroxy DBA was investigated in the urine of rabbits, rats, and monkeys. The ether fractions obtained after hydrolysis and autoclaving of the urine did not yield additional DBA or phenolic derivatives as judged by the spectroscopic evidence. If phenolic derivatives of DBA were conjugated as glucuronides or as ethereal sulfates, the ether-soluble free compounds should have been present in these fractions. Occasionally, traces of the phenolic derivatives were observed after hydrolysis of the urine so that conjugation of this type cannot be entirely excluded. The possibility also exists that unstable ethereal sulfates or glucuronides are formed which hydrolyze immediately after acidification of the urine or during the extraction procedures. Boyland and Levi (5) have presented evidence of an increased excretion of ethereal sulfates following the feeding of DBA to rabbits. However, there is no proof that the increase is due to a conjugated DBA derivative. It is possible that, because of the toxic action of DBA, an increase of ethereal sulfates may result from substances other than metabolites of this hydrocarbon.

The possibility of the formation of a DBA mercapturic acid was investigated. Since mercapturic acids are rarely ether-soluble, chloroform and butyl alcohol extractions of rabbit and rat urines were made. No indications, either chemical or spectroscopic, of a DBA mercapturic acid were observed. Furthermore, following the hydrolysis or autoclaving procedures, no free DBA was observed spectroscopically. From the data, it appears that the formation of conjugated derivatives of DBA or its hydroxy derivatives is not one of the principal metabolic pathways for the handling of DBA.

Aromatic unsubstituted hydrocarbons are hydroxylated in many instances in the course of their metabolism. The symmetrical configuration of the phenolic dihydroxy conversion product excreted by rats is an interesting metabolic phenomenon. Hydrocarbons of more than one ring system are metabolized to mono-hydroxylated derivatives (naphthalene) or to dihydroxy dihydro derivatives in neighboring positions (anthracene). Boyland and Levi (2, 3) showed that rabbits and rats fed with anthracene excrete optical isomers of 1,2-dihydroxy-1,2-dihydroanthracene. Wiley (25) gave evidence for the hydroxylation of

β-naphthylamine in neighboring positions in dogs, whereas Dobriner, Hofmann, and Rhoads (13) have shown that rats, rabbits, and monkeys excrete a β-naphthylamine metabolite which is hydroxylated in the 6- or symmetrical position. How far species differences are responsible for hydroxylation in neighboring or symmetrical positions remains to be demonstrated.

Although only a few studies on the metabolism of carcinogenic hydrocarbons are available, only in the case of DBA has a phenolic derivative been isolated. Evidence indicates that methylcholanthrene (15) 3,4,5,6-dibenzcarbazole (5) and benzpyrene (9, 10) probably are metabolized to phenolic compounds. The primary formation of alcoholic dihydroxy dihydro derivatives in neighboring positions and the secondary splitting off of water with the formation of a phenol as possibly in the case of naphthalene metabolism, must be considered. Furthermore, the biochemical and biological significance of the formation *in vivo* of phenolic derivatives of carcinogenic hydrocarbons must be established. Some information is available in regard to the carcinogenic activity of synthetic phenolic and alcoholic derivatives of very active carcinogens. These compounds show very little or no carcinogenic activity (1, 17, 16, 23, 24, 18-20).

The difference in metabolism of DBA among various species which results in the production of dihydroxy derivatives of different configuration is of great interest not only from the metabolic point of view but also from the point of view of carcinogenic activity.

Since DBA produces tumors in mice (11), and to a lesser extent in rats, and not at all in rabbits (6), it is of interest to know whether the carcinogenic properties of DBA are dependent upon the intermediary metabolism or upon the final conversion of this compound. This investigation has demonstrated that various species metabolize DBA differently and has raised the question of the carcinogenic power of the DBA metabolites produced by the different species.

The phenolic derivative produced by rabbits was injected into mice and was found to be noncarcinogenic. A control experiment in which DBA was injected in the same amount into litter mates of the mice showed that this strain of mice was highly susceptible to tumor formation caused by DBA. The phenolic derivative produced by the rabbit was injected only into a small number of mice. A larger series of animals and the administration of larger amounts is desirable. Boyland tested 10 mice by painting the skin twice weekly with the dihydroxydibenzanthracene derivative which he isolated from rabbits. He also injected 10 mice twice weekly with 1 mgm. each of the phenolic derivative. None of the mice showed tumor formation, thus confirming the findings of this group. It will be of great interest to know whether the phenolic derivative produced by rats and mice will have the same lack of carcinogenic activity.[4] The decrease or lack of carcinogenic power of synthetic hydroxylated hydrocarbons has been discussed previously. There is no doubt that both *in vivo* and *in vitro,* hydroxylation renders strong carcinogens less or noncarcinogenic. The question still remains as to why the carcinogenic potency of the same hydrocarbon is different in different species. There is some evidence that only a very small amount of DBA injected in olive oil is metabolized to a noncarcinogenic phenolic derivative. A large amount of the DBA remains in the animal. Lorenz and Stewart (22) have reported that DBA can be recovered only in very small amounts from the feces of mice which have been fed the hydrocarbon. Studies are in progress which, it is hoped, will provide more information concerning the qualitative and quantitative aspects of DBA metabolism in different species.

SUMMARY

1. By a combination of chemical fractionation and qualitative spectroscopic methods the fate of dibenzanthracene was investigated in the excreta and certain tissues of mice, rats, rabbits, dogs, and monkeys.

2. The spectroscopic investigations gave evidence for the presence of phenolic derivatives of dibenzanthracene in the excreta of mice, rats, rabbits, and dogs. No metabolite of dibenzanthracene was suggested in the excreta of monkeys.

3. The spectroscopic investigation suggested that the phenolic derivatives of dibenzanthracene are identical in mice and rats and differed from the phenolic metabolite excreted by rabbits.

4. No evidence was obtained for the excretion of a conjugated derivative of dibenzanthracene in any of the species studied.

5. Small amounts of dibenzanthracene are excreted in the urine and feces of mice, rats, and rabbits.

6. A preliminary quantitative estimation of the comparative ability of different species to convert dibenzanthracene shows that mice and rats convert only a small amount of the injected dibenzanthracene to the phenolic derivative as compared to rabbits.

7. The phenolic derivatives of dibenzanthracene excreted by mice, rats, and rabbits were isolated. The phenolic metabolites of mice and rats are identical with 4′,8′-dihydroxydibenzanthracene synthesized by Fieser and Cason.

[4] Since this manuscript was submitted, Dunlap and Warren (Cancer Research, **1**:953-954. 1941.) have reported that 4′,8′-dihydroxy-1,2,5,6-dibenzanthracene, the phenolic derivative excreted by rats and mice, did not produce tumors in mice when tested over a period of 8 months.

8. The dihydroxydibenzanthracene produced by rabbits did not show carcinogenic activity when injected in mice.

9. The chemical and biological significance of the conversion of dibenzanthracene to phenolic derivatives is discussed.

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Foster Nursing and the Growth of Transplantable Leukemias in Mice

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The observations of many investigators relating to the transplantability of spontaneous sarcomas and carcinomas and of induced sarcomas have established the fact that malignant tissues arising in inbred strains of mice are usually transplantable only to mice of the same inbred strain, to F_1 hybrids including that strain, and to a lesser extent to later hybrid generations. Transplantable leukemia has been shown to behave in like manner. From the work reported, the conclusion has been reached that susceptibility or nonsusceptibility to malignant tissue is dependent upon the relationship existing between the genetic constitution of the host and of the tumor cell.

The induction of resistance to transplantable tumors in mice by a prior inoculation of homologous normal living cells was early reported by Bashford, Murray, and Cramer (3). Numerous subsequent studies of resistance and susceptibility to transplantable tumors were reviewed by Woglom (17) in 1929. More recently Barrett (2) has shown that defibrinated blood induced some resistance in susceptible mice to the growth of transplantable tumors. In these cases the phenomenon involved seems to be a type of immunization directed against intact cells and depending upon a degree of genetic difference between the cells injected and the host.

Rhoads and Miller (16) showed that resistance to transplantable leukemia may be induced by injection of intact homologous cells; and MacDowell, Potter, and Taylor (14) showed that "immunity" to transplantable leukemia could be induced by (a) inoculations of small doses of leukemic cells followed by increasingly larger doses, or by (b) inoculations of embryo or adult homologous tissues.

On the other hand, very few reports have appeared indicating establishment of susceptibility in resistant hosts or the breaking down of resistance to the transplantation of tumors from alien strains. Mechanical irritation was found by Jones (8) to increase the susceptibility of resistant mice to the growth of an adenocarcinoma. A piece of sterile, non-dyed flannel was used and was inoculated with the tumor. Irradiation of mice by sublethal doses of x-rays has produced a definite increase in susceptibility of mice to transplantable neoplasms. Murphy and co-workers (15), who discovered this effect, attribute the change in susceptibility to the destructive action of x-rays on the lymphatic tissues. This procedure has been used extensively by Furth (7) and his associates in studying the characteristics of transplantable leukemias. Andervont (1) found that injection of trypan blue into the host lowered the resistance of mice to transplantation of tumors from other strains. Lewis and Lichtenstein (11-13) have shown that a few tumors of a large series of induced malignancies were found to grow in refractory mice following repeated inoculations of tumors of alien strains. Some of these tumors were then found to grow progressively in other alien strains.

* Medical Research Fellow of The Commonwealth Fund.

MATERIALS AND METHODS

An active influence transmitted through the mother's milk is known to affect the incidence of mammary carcinoma in mice (4). Thus it was thought worthwhile to extend this work and study the effect of foster nursing on the growth of transplantable tumors. The present report concerns the effects of foster nursing on the growth of 3 transplantable leukemias.

The mice used were from the dilute brown (Dba) strain, derived as follows: Four distinct segregating sublines of the dilute brown Dba stock have been carried at this laboratory by Dr. G. W. Woolley. Each of these sublines, designated as sublines 1, 2, 3, and 4, has been carried for 30 generations by brother to sister matings. Apparent differences exist in mammary carcinoma incidence, spontaneous leukemia incidence, and breeding behavior between some of these sublines. Within subline 1 two distinct lines have segregated and are known as sublines 111 and 122. Only subline 122 has been used in this study and these animals will be designated Dba subline 1. Within subline 2 two distinct segregating lines have appeared and are called sublines 212 and 214. Only subline 212 mice have been used in this study and these will be referred to in the text as Dba subline 2 mice. Transplantation studies at this laboratory have shown that tumors arising in sublines 2, 3, or 4 can be transplanted to mice of any of these 3 sublines with 100 per cent takes. Consequently in a few instances subline 3 and subline 4 mice have been used interchangeably with subline 2 mice without reference to such in the text.

All 3 leukemias arose in the Dba strain of mice. Two of the leukemic lesions (LL449 and LL493) were induced by the highly potent carcinogen 9,10-dimethyl-1,2-benzanthracene (9). Lymphoid leukemia LL449 was produced in subline 212 and myeloid leukemia LL493 arose in subline 111. The third leukemia studied, P1534, appeared spontaneously in subline 3 of the Dba strain as a lymphosarcoma.

It was transplantable through the first 4 generations as such after which it became systemic and acquired the characteristics of a lymphoid leukemia.

A description of leukemia line LL449 has been given in a preliminary report on the effect of foster nursing on the growth of this tumor (10).

Leukemia LL493 appeared in ♀D17, 172 days following initial painting with 9,10-dimethyl-1,2-benzanthracene. The animal appeared moribund and emaciated and was killed *in extremis* 2 days later. The spleen was greatly enlarged, pale and grayish, and nodular on cut surface. The liver was slightly enlarged and pale. The mediastinal and mesenteric-intestinal lymph nodes were greatly enlarged. Subcutaneous lymph nodes were slightly enlarged and some showed hemorrhagic spots. There was severe infiltration in the kidneys. The cardiac leukocyte count was 18,900 cells per cu. mm. The following differential count was obtained: lymphocytes 36 per cent, neutrophils 32 per cent, monocytes 3 per cent, eosinophils 2 per cent, and immature granulocytes 27 per cent with nearly equal distribution of myeloblasts and myelocytes which were peroxidase positive. The blood picture has remained materially the same in 13 transfer generations.

Histologically the architecture of the spleen was almost entirely obliterated with infiltration of mature and immature granulocytes in the red pulp. Megakaryocytes were noticeably increased. There occurred extensive infiltration of mature and immature granulocytes in the sinusoids of the liver. Infiltration was noted in the affected lymph nodes. This was extreme chiefly in the medullary regions. Numerous plasma cells were present.

Leukemia P1534 arose as a spontaneous lymphosarcoma involving only the mediastinal lymph node. This leukemia appeared at 339 days in ♀8495 in subline 3 of the Dba strain of Dr. G. W. Woolley. This animal had been ovariectomized at birth. The tumor transplanted as a lymphosarcoma during the first 3 transplant generations. In the 9th transplant generation systemic infiltrations occurred into the spleen, lungs, adrenals, kidneys, and mesenteric-intestinal lymph nodes. Histologically the infiltrating cells were medium and large lymphocytes. A slight elevation of the lymphocyte count occurred, also a high white blood cell count. In the subsequent 9 transfer generations the leukemia has remained systemic.

Inoculations of pieces of neoplastic tissue, approximately 1 cu. mm. in size, were made subcutaneously into the right axilla by use of a sterile trocar. Non-necrotic tissue taken from subcutaneous growths of the neoplasm was used throughout. All negative animals were kept for 6 months. Inoculations were made into mice one month old.

The usual procedure in foster nursing was to transfer a litter to the foster mother within 24 hours after birth. The foster mothers used, however, were at various stages of the lactation period. Mice were thus fostered until weaning age. Equal numbers of males and females were used. Since no sex differences in the growth of tumors were observed, the sexes are combined in all tabulations.

Leukemia Line LL449

A preliminary report on the growth of this strain of lymphoid leukemia has been published (10). A total of 521 mice has been used in the present study of the growth of this tumor. This leukemia has been carried through 13 transplant generations and has grown 100 per cent in susceptible Dba mice of subline 2 and the closely allied sublines 3 and 4. On the other hand, it has failed to grow in mice of subline 1 of the Dba strain (53 negative out of 54 mice), in the C57 black, C3H, C, A^w, and Swiss strains following identical inoculations.

If mice of the resistant strains are foster nursed by mothers of the susceptible strain, Dba subline 2, there results in some of the fostered animals growth of this leukemia following a single inoculation (Table I). Four types of reaction were observed in resistant fostered mice following transplantation of leukemia LL449, as follows: 1. No growth or a slight swelling to the size of a pea probably due to accumulation of leukocytes. These are considered negative (−) mice. 2. Extreme growth *sub cutem* ($>2.5\times2.5$ cm.) of the inoculum similar to the growth of lymphosarcoma cells. There was no systemic involvement but in some animals an escape of some malignant cells into the blood stream occurred. Histologically these subcutaneous growths showed typical neoplastic cells and were transplantable 100 per cent to the susceptible Dba 2 subline. In many cases total regression occurred after 3 to 4 weeks. These growths are referred to in the tables as (+). 3. The third type of reaction was similar to the second. However, along with a subcutaneous growth of the inoculum, connective tissue edema, distention of the abdomen, and a general ruffling of the hair occurred. Although macroscopically there was no pronounced infiltration into the spleen, liver, kidneys, and lymph nodes, microscopically these organs showed a moderate amount of infiltration. In animals showing this type of reaction more lymphoid cells escaped into the blood. In spite of the advanced stage of growth in a few of these animals an apparent complete regression resulted in 3 to 4 weeks. This type of reaction is referred to in the tables as (++). 4. Subcutaneous and systemic growth of the leukemia resulted in death of the animals. There was little or no delayed appear-

ance of the lesion and the clinical course of the disease as determined by invasion of leukemic cells, blood counts, and length of life following inoculation was similar to that occurring in mice of the susceptible strain. These animals are designated as (+++).

The type and extent of growth of leukemic cells in resistant and resistant-fostered mice following inoculation were apparently influenced by the strain of the donor. Dba subline 1 mice were nearly 100 per cent resistant to leukemic cells when tissue from mice of Dba subline 2 was inoculated. On the other hand, when leukemic cells which grew in resistant subline 1 mice fostered on susceptible subline 2 mice were used as the inoculum, 91.4 per cent of the resistant Dba subline 1 mice grew the leukemia. Mice of subline 1 fostered by susceptible subline 2 mothers reacted similarly to nonfostered subline 1 mice following inoculation of leukemic cells obtained from resistant or resistant-fostered donors.

In like manner, there occurred a higher percentage of takes and a difference in the type of growth in C57 black mice fostered on susceptible Dba subline 2 mice when leukemic cells from resistant Dba subline 1 fostered mice were used as the inoculum (Table I).

Table I: Reactions of Normal and Foster-Nursed Mice to Transplantable Lymphoid Leukemia Line LL449

Donor—leukemia cells obtained from	Recipient	Number of animals	Reaction *	Per cent positive
Dba subline 2	Dba subline 2	63	61+++, 2—	96.8
	Dba subline 1	54	1+++, 53—	1.9
	C57 black	46	46—	0.0
	Dba subline 1 fostered by Dba subline 2	36	11+++, 3++, 3+ (2 regressions), 19—	47.2
	C57 black fostered by Dba subline 2	24	6+++, 18—	25.0
Dba subline 1 fostered by Dba subline 2.	Dba subline 2	31	31+++	100.0
	Dba subline 1	39	18+++, 14++ (5 regressions), 7—	82.4
	C57 black	46	46—	0.0
	Dba subline 1 fostered by Dba subline 2	19	5+++, 9++, 5—	73.6
	C57 black fostered by Dba subline 2	39	3++, 16+ (10 regressions), 20—	48.7
	Swiss	14	14—	0.0
	Swiss fostered by Dba subline 2	8	5+ (4 regressions), 3—	62.5
	C3H	15	15—	0.0
	C3H fostered by Dba subline 2	15	3+++, 8++ (6 regressions), 4—	73.3

* See text for explanation of reaction types.

Leukemia Line P1534

This spontaneous lymphoid leukemia has been transplanted 100 per cent in mice of the strain of origin Dba subline 3 or in the closely allied subline 2. A large subcutaneous growth occurs with infiltration into regional lymph nodes, infiltration into spleen, liver, and in most advanced cases into the lungs and kidneys. There is a moderate escape of lymphoid cells into the blood stream. Growth of this neoplasm in subline 1 of the Dba strain has occurred in only 23 of 103 animals, while no growth has been observed in mice of the C57 black or Swiss strains. Following foster nursing of these refractory strains by mothers of the Dba subline 2 strain, growth of leukemic cells has resulted in some mice, although the type of growth observed has not always had the characteristics of that observed in the susceptible strain. Subcutaneous growth only of inoculated leukemic cells similar to growth of lymphosarcoma cells has resulted in some mice (Table II).

As in lymphoid leukemia line LL449, leukemic cells of line P1534 appear to have different growth potentialities depending upon the type of donor of the leukemic cells. This leukemia has been carried for 13 transplant generations in Dba subline 2 mice. During this period 22.3 per cent (23 of 103 mice) of the refractory Dba subline 1 mice grew the neoplasm. Leukemic cells which grew in refractory Dba subline 1 mice following fostering on susceptible mothers (constituting transplant generations 8 to 13) produced 71.0 per cent (71 of 100 animals) takes in refractory Dba subline 1 mice.

Leukemia Line LL493

This myeloid leukemia has been carried for 15 transfer generations in subline 1 of the Dba strain, the strain of origin. During this period it has produced nearly 100 per cent takes (144 of 147 mice). Dba subline 2 mice have proved nearly totally refractory to the leukemic cells (35 negative of 36 mice). No growth has been observed in mice of the C57 black strain.

Refractory Dba subline 2 or C57 black mice foster

nursed by mothers of the susceptible Dba subline 1 strain did not prove susceptible to inoculations of leukemic cells.

The same change in growth capacity of leukemic cells as observed for tumors LL449 and P1534 which grew in fostered alien strain mice was noted for this neoplasm. Although subline 2 of the Dba strain was nearly totally refractory to leukemic cells from donor Dba subline 1 animals, 37.5 per cent (39 of 104 mice) grew this neoplasm when the donor animals were either fostered subline 2 mice or subline 2 mice which grew the neoplasm in serial transfer following initial

In each serial transfer of the lymphoid leukemia LL449, in refractory Dba subline 1 hosts and in refractory C3H hosts, an apparent decrease in growth vigor of the neoplasm has resulted such that later serial transfers either fail to grow or grow only locally with a final complete regression. In contrast, cells of leukemias LL493 and P1534 produce the same type and extent of growth in late serial transfer generations in refractory hosts as in early generations. Myeloid leukemia LL493 has been carried through 7 serial transfer generations without loss of growth potentiality in refractory Dba subline 2 hosts (Table IV).

TABLE II: REACTIONS OF NORMAL AND FOSTER-NURSED MICE TO TRANSPLANTABLE LYMPHOID LEUKEMIA LINE P1534

Donor—leukemia cells obtained from	Recipient	Number of animals	Reaction *	Per cent positive
Dba subline 2	Dba subline 2	79	79+++	100.0
	Dba subline 1	103	17+++, 6+ (1 regression), 80—	22.3
	Dba subline 1 fostered by Dba subline 2	70	43+++, 2+ (2 regressions), 35—	64.5
	C57 black	40	40—	0.0
	C57 black fostered by Dba subline 2	38	5+++, 1+ (regression), 32—	15.7
	Swiss	14	14—	0.0
	Swiss fostered by Dba subline 2	18	1+++, 2+, 15—	16.7
Dba subline 1 fostered by Dba subline 2.	Dba subline 2	23	23+++	100.0
	Dba subline 1	100	62+++, 9+ (7 regressions), 29—	71.0
	Dba subline 1 fostered by Dba subline 2	18	10+++, 5+ (3 regressions), 3—	83.3

* See text for explanation of reaction types.

TABLE III: REACTIONS OF NORMAL AND FOSTER-NURSED MICE TO TRANSPLANTABLE MYELOID LEUKEMIA LINE LL493

Donor—leukemia cells obtained from	Recipient	Number of animals	Reaction *	Per cent positive
Dba subline 1	Dba subline 1	147	144+++, 3—	91.2
	Dba subline 2	36	1+++, 35—	2.8
	Dba 2 fostered by Dba 1	48	1+++, 47—	2.1
	C57 black	34	34—	0.0
	C57 black fostered by Dba 1	58	58—	0.0
Dba 2 fostered by Dba 1.	Dba subline 1	86	83+++, 3—	96.5
	Dba subline 2	104	23+++, 16— (8 regressions), 65—	37.5

* See text for explanation of reaction types.

growth in the fostered animal. Sixteen of the 39 positive subline 2 animals grew the neoplasm only subcutaneously with little or no infiltration into organs. Eight of these growths subsequently regressed but it is to be noted that all such growths were readily transplantable to the susceptible subline 1 mice and to a lesser extent to subline 2 mice (Table III).

SERIAL PASSAGE OF LEUKEMIC CELLS IN REFRACTORY HOSTS

When leukemic cells from any of the lesions studied grow progressively in refractory mice fostered by susceptible mothers, these malignant cells can be carried by serial passage in mice of the refractory strain without further resort to foster nursing. The data of such experiments are presented in Table IV.

Transplantation of leukemic cells which grew progressively in refractory mice back to the strain of origin results in the loss of this capacity to grow in the refractory strain. This phenomenon was observed in only one of the transplantable leukemias.

In lymphoid leukemia P1534 transplantation was made from Dba subline 1, a normally refractory strain in which 26 of 33 mice grew the neoplasm progressively, back to Dba subline 2 mice. The leukemic cells were carried through 3 transfer generations in subline 2 in which 100 per cent takes occurred. Transplantation was then made to 16 refractory Dba subline 1 mice with no resultant takes (Table IV).

Although cells of leukemias LL449 and P1534 grew in a few mice of the C57 black strain which were fostered by mothers of the Dba subline 2, inocula-

tions of leukemic cells from C57 black donors failed completely to grow in C57 black mice (25 mice for LL449 and 20 mice for tumor P1534). Inoculation of identical tissue into Dba subline 2 mice produced 100 per cent takes.

Leukemic cells which grew in refractory mice produced the same clinical picture when reinoculated into the susceptible strains as did leukemic cells carried by serial passage within the susceptible strain.

There was no loss of strain specificity of any of the three transplantable leukemias studied such as that observed by Lewis and Lichtenstein (11-13) whereby tumors which grew in one alien strain as a result of successive inoculations were found to grow in other

TABLE IV: SERIAL PASSAGE OF LEUKEMIC CELLS IN MICE OF REFRACTORY STRAINS FOLLOWING GROWTH IN REFRACTORY FOSTER-NURSED MICE

Strain of leukemia	Generation transfer to refractory mice	Generation of transplant	Donor	Recipient	Reaction *
LL449	G6	G1	Dba 2	3 Dba 1 fostered by Dba 2	2+++, 1—
		G2	Dba 1 fostered by Dba 2	6 Dba 1	4+++, 2+ (Regression)
		G3A	Dba 1	11 Dba 1	9+++, 2+ (Regression)
		G3B	Dba 1	6 Dba 2	6+++
		G4	Dba 1	12 Dba 1	5+++, 7+ (Regression)
		G5	Dba 1	10 Dba 1	2+++, 1+ (Regression), 7—
LL449	G4	G1	Dba 2	5 Dba 1 fostered by Dba 2	5+++
		G2	Dba 1 fostered by Dba 2	7 Dba 1	7—
LL449	G11	G1	Dba 2	5 C3H fostered by Dba 2	2+++, 3++
		G2	C3H fostered by Dba 2	9 C3H	9+++
		G3A	C3H	10 C3H	4+++, 6++ (Regression)
		G3B	C3H	3 Dba 2	3+++
P1534	G9	G1	Dba 2	11 Dba 1 fostered by Dba 2	10+++, 1—
		G2	Dba 1 fostered by Dba 2	25 Dba 1	18+++, 7—
		G3	Dba 1	12 Dba 1	8+++, 4—
		G4	Dba 1	3 Dba 2	3+++
		G5	Dba 2	4 Dba 2	4+++
		G6	Dba 2	5 Dba 2	5+++
		G7a	Dba 2	3 Dba 2	3+++
		G7b	Dba 2	16 Dba 1	16—
LL493	G8	G1	Dba 1	7 Dba 2	1+++, 6—
		G2	Dba 2	5 Dba 1	2+++, 3—
			Dba 2	10 Dba 2	4+++, 1+ (Regression), 5—
		G3	Dba 2	10 Dba 1	10+++
		G3	Dba 2	10 Dba 2	2+++, 8—
			Dba 2	19 Dba 2	1+++, 4+, 14—
			Dba 2	22 Dba 1	22+++
		G4	Dba 2	21 Dba 2	5+++, 3+ (Regression), 13—
			Dba 2	16 Dba 1	16+++
		G5	Dba 2	5 Dba 2	1+++, 2+ (Regression), 2—
				5 Dba 1	5+++
		G6	Dba 2	14 Dba 2	5+++, 9—
		G7	Dba 2	12 Dba 2	3+++, 2+ (Regression), 7—
			Dba 2	6 Dba 1	6+++
LL493	G9	G1	Dba 1	8 Dba 2 fostered by Dba 1	1+++, 7—
		G2	Dba 2	12 Dba 1	12+++
				8 Dba 2	1+++, 4+, 3—
		G3	Dba 2	6 Dba 1	6+++
				5 Dba 2	1+++, 2++, 2—
				5 Dba 1	5+++

* See text for explanation of reaction types.

alien strains. No growth of strain LL493 or strain LL449 leukemia was observed in mice of the A, C, Swiss, and BBC strains when leukemic cells which grew in refractory foster-nursed mice or leukemic cells carried by serial passage in refractory mice were used as the inoculum.

THE TIME INTERVAL IN FOSTER NURSING AND THE SUSCEPTIBILITY AGENT

The influence present in the milk which induces susceptibility in some refractory mice to certain transplantable leukemias was found to be present in the milk secreted by lactating females at various times up to the 10th day of the lactation period.

Refractory mice which have nursed their own mother for as long as 6 days prior to foster nursing have been rendered susceptible to inoculated leukemic cells following foster nursing on susceptible females (Table V).

Table V: Effect of Time Interval on the Reaction of Foster-Nursed Mice to Growth of Transplantable Leukemias

Line of leukemia	Strain of mice	Fostered by	Time interval *	Number of days fostered	Number of mice	Reaction †
LL449	C57 black	Dba 2	12 hours	21 days	5	2++
	Dba 1	Dba 2	12 hours	21 days	3	2++
	C57 black	Dba 2	6 days	15 days	3	3+
	C57 black	Dba 2	12 hours	24 hours	5	2+ subsequent regression
	C57 black	Dba 2	24 hours	21 days	3	2+++
	C57 black	Dba 2	5 days	16 days	8	4+ subsequent regression
	Dba 1	Dba 2	2 days	19 days	12	6+++
	Dba 1	Dba 2	12 hours	21 days	5	5+++
	C57 black	Dba 2	12 hours	21 days	6	6+++
	Dba 1	Dba 2	12 hours	21 days	6	2+++, 2++ subsequent regression
	C57 black	Dba 2	6 days	15 days	3	3+
	C57 black	Dba 2	5 days	16 days	7	4+
P1534	Dba 1	Dba 2	7 days	14 days	4	4+++
	Dba 1	Dba 2	6 days	15 days	6	4+++
	Dba 1	Dba 2	24 hours	21 days	5	5+++
	Dba 1	Dba 2	2 days	19 days	10	10+++
	Dba 1	Dba 2	4 days	17 days	4	2+++

* Time interval = time between birth and foster nursing.
† See text for explanation of reaction types.

Mice of the susceptible strain which have been removed from their mother within 12 hours following parturition or mice born of mothers with sealed nipples and foster nursed by females of the refractory strains proved 100 per cent susceptible to inoculations of leukemic cells (Table VI).

Second generation mice obtained by mating litter mates of refractory foster-nursed mice proved in some cases to be susceptible to inoculations of leukemic cells. The reaction observed in second generation mice inoculated with leukemic cells of strain LL449 was a temporary local growth only, with subsequent regression in all mice. This was not the characteristic type of reaction observed in straight foster-nursed mice. On the other hand, 14 of 31 second generation mice inoculated with leukemic cells of lymphoid leukemia P1534 gave the typical extreme generalized reaction produced in Dba subline 2 mice (Table VII). There were 45.1 per cent positive second generation mice as compared with 64.5 per cent positive of the straight fostered mice. Later generations have not as yet been tested in order to establish whether or not this influence is similar to the "milk-influence" active in spontaneous mammary carcinoma in being transmitted to successive generations by breeding (5).

Table VI: Reaction of Susceptible Mice Fostered on Resistant Strains to Growth of Transplantable Leukemias

Line of leukemia	Strain of mice	Fostered by	Number of mice	Reaction †
LL449	Dba 2	Dba 1	5	5+++
	Dba 2 *	Dba 1	4	4+++
	Dba 2 *	C57 black	5	5+++
	Dba 2 *	C57 black	5	5+++
	Dba 2	C57 black	5	5+++
	Dba 2 *	C57 black	4	4+++
P1534	Dba 2	Dba 1	6	6+++
	Dba 3	C57 black	5	5+++
	Dba 2	C57 black	6	6+++
LL493	Dba 1	C57 black	4	4+++
	Dba 1	Dba 2	6	6+++
		Totals . . .	55	55+++

* Mice born of mothers with sealed nipples.
† See text for explanation of reaction types.

DISCUSSION

The results presented in this paper indicate the presence in the milk of female mice susceptible to the growth of leukemic cells of lines LL449 and P1534 of an influence which may lower the genetically controlled resistance to the growth of these malignant

cells in refractory mice which have been foster nursed by mice of the susceptible strain. Another extrachromosomal influence exerted probably through the uterus or the milk on mice derived from transferred ova has been reported by Cloudman (6) to affect the growth of a transplantable fibrosarcoma and a melanoma.

The nature of this influence is not yet known. Apparently it is present in the milk of susceptible females during most of the lactation period. Preliminary results indicate that the influence cannot be transferred by tissue inoculations into young refractory mice. This is to be expected in view of the degree of "immunity" to transplantable leukemia resulting from homologous tissue inoculations (14, 16).

Experiments are now under way to test whether or not a similar agent exists in the milk of other inbred strains of mice affecting the growth of other types of transplantable tumors. This agent has been active only as regards transplantable lymphoid leukemia. No such factor was evident in influencing the growth of myeloid leukemia, line LL493. Preliminary evidence indicates that foster nursing does not influence the growth of an induced monocytic leukemia nor of an induced fibrosarcoma.

The phenomenon concerned with the change in growth capacity of leukemic cells of all 3 tumors investigated, whereby these malignant cells grow in serial transfer in refractory mice (of Dba subline 1 and C3H strains) following an initial growth in refractory foster-nursed mice, does not appear to be a change in the leukemic cells. Malignant cells from refractory donor mice in which these cells grew give the same clinical picture and same type and extent of growth when reinoculated into the susceptible strain as do leukemic cells carried by serial transfer within the susceptible strain. The fact that the capacity of leukemic cells (of line P1534) to grow by serial transfer in refractory mice is lost when the malignant cells are reinoculated into the susceptible strain indicates that the factor involved must arise from within the refractory mice.

The mechanism whereby the degree of immunity against inoculations of leukemic cells is reduced in refractory mice which have ingested milk from mice which grow the leukemic cells remains unexplained. It appears, however, that the mechanism involved is a systemic, or general, rather than a local, process.

SUMMARY

The degree of resistance against progressive growth of inoculated malignant cells characteristic of certain refractory strains of mice has been reduced by foster nursing of the refractory mice by mothers of the susceptible strain. The influence present in the milk of Dba subline 2 mice has been shown to affect the growth of two transplantable lymphoid leukemias, line LL449 and line P1534. On the other hand, foster nursing was shown to be ineffective in influencing the growth of a myeloid leukemia, line LL493.

The "susceptibility" influence apparently is present in the milk of lactating females during the first 6 or 7 days of the lactation period. Data are not as yet available to determine the presence or effectiveness of the susceptibility influence during the latter part of the lactation period. Total deprivation of susceptible milk in young of the susceptible strain followed by foster nursing by females of the refractory strain does not affect the response of these mice to inoculations of leukemic cells.

A change in the growth capacity of the malignant cells of the two lymphoid leukemias, line LL449 and line P1534, and of the myeloid leukemia, line LL493, whereby leukemic cells can be grown by serial transfer

TABLE VII: REACTION OF OFFSPRING OF REFRACTORY FOSTER-NURSED MICE TO GROWTH OF TRANSPLANTABLE LEUKEMIAS

Line of leukemia	Strain of mice	Fostered by	Generation	Number of mice	Reaction *
LL449	Dba 1	Dba 2	G2	3	3+ subsequent regression
	C57 black	Dba 2	G2	3	3—
	C57 black	Dba 2	G2	4	3—, 1+ subsequent regression
	C57 black	Dba 2	G2	3	3+ subsequent regression
	C57 black	Dba 2	G2	5	4—, 1+ subsequent regression
			Totals..	18	8+, 10—
P1534	Dba 1	Dba 4	G2	3	2+++, 1—
	Dba 1	Dba 4	G2	8	3+++, 5—
	Dba 1	Dba 2	G2	12	9+++, 3—
	Dba 1	Dba 4	G2	3	3—
	Dba 1	Dba 4	G2	5	5—
			Totals..	31	14+++, 17—

* See text for explanation of reaction types.

in refractory mice has been observed. This occurs following initial growth of the malignant cells in refractory foster-nursed mice.

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Character of Changes Occurring in the Course of Transplantation of Two Strains of Lung Tumors in Mice*

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Serial transplantation of adenomas of the lung of mice by the subcutaneous route has been described recently by Andervont (1, 2). In the course of successive passages 6 transmissible tumors retained the histological structure of the original growth, 7 lost the characteristic adenomatous structure to a considerable extent, but the tumors still appeared to be epithelial in character, while 5 changed into spindle-cell tumors. Both spontaneous and induced lung tumors exhibited this remarkable phenomenon of transformation in the course of successive subcutaneous passages from adenomatous into spindle-shaped structures.

Andervont mentions several possibilities to explain this phenomenon: (a) the lung tumors arise as mixed tumors and the sarcoma cells overgrow the carcinoma cells in the subcutaneous tissues of the new host; (b) the stromal cells supplied by the animal bearing the transplanted tumor become sarcomatous; (c) the malignant epithelial cells change in appearance; (d) the primary tumors are not epithelial in character. Campbell (4) describes the presence of spindle-shaped cells in primary lung tumors and believes that there is evidence of a change of epithelial cells into this type of cell. Wells, Slye, and Holmes (8) found that 33 of 104 metastatic tumors from spontaneous lung tumors of mice showed more or less the structure of a sarcoma. This was true even when no sarcomatous elements were seen in the primary growth. The sarcomatous structure was, however, evident in a number of primary lung tumors. In their opinion, this is a true spindle-cell growth with collagenous fibers between the cells, and not the oat-cell growth of undifferentiated lung carcinoma. The histological appearance of the original lung tumors observed by Andervont (2) did not show sarcoma in any case and his investigations did not elucidate the nature of this remarkable transformation of adenoma of the lung of the mouse into spindle-cell tumors.

The present investigations were undertaken to obtain information concerning the character of this morphological change. Previous studies on the histogenesis of spontaneous and induced lung tumors made in this laboratory (5) led to the conclusion that they originate in the lining cells of the alveoli, an opinion reached independently by several investigators (6). Preceding the experiments now to be described the histogenesis of induced lung tumors was studied on material obtained from animals killed at various intervals in the course of painting with methylcholanthrene. The results of these investigations confirm those previously reported (6) and since most workers now agree that induced tumors originate in the lining cells of the alveoli, these studies will not be described.

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MATERIAL AND METHODS

All mice used were bred in the laboratory and unless otherwise stated were approximately 5 weeks of age. Stock Ak is an inbred high-leukemia stock with an incidence of leukemia of approximately 70 per cent, and 1 per cent of lung tumors. Stock Rf is a low-leukemia stock with an incidence of lymphoid and myeloid leukemia of 2 per cent, monocytic leukemia 2.7 per cent, and lung tumors over 32 per cent.

In F_1 hybrids between these two stocks the incidence of lung tumors is approximately 19 per cent, in F_2 hybrids 17.5 per cent, and in F_3 hybrids 12 per cent.

Mice that died within 20 days after injection and showed no evidence of tumor were discounted from the total number of mice injected.

Unless otherwise stated tumors grown in the subcutaneous tissue were used for transfer. The growth was finely minced in Tyrode's solution and the particles injected subcutaneously or intraperitoneally through an 18 or 20 gauge needle. Material for intravenous inoculation was obtained by allowing the tumor suspension to settle for a few seconds and then drawing off the supernatant through a 27 gauge needle.

Lung Tumor Strain A

Origin.—An F_2 hybrid mouse (Ma 387), killed at 450 days of age, had several tumors in the lung varying in size from 1 to 2 mm. in greatest diameter. These were excised under aseptic conditions, cut up in fine particles, and injected into the subcutaneous tissue and peritoneal cavity of each of 4 mice, 45 days of age. Two of these, killed 131 days after injection, were free from tumors and the other 2 had tumors at the site of inoculation, each measuring 6 mm. in greatest diameter. A subpassage from one of these was successful in 5 of the 6 injected mice. Subsequently 16 successive passages were made, the results of which are summarized in Table I.

TABLE I: TRANSMISSION EXPERIMENTS WITH LUNG TUMOR STRAIN A

Passage	Material injected	Stock of mice injected	Route of injection *	Number of mice injected	Number with lung tumor	Number with no lung tumor	Number negative
I	Lung tumor	F_2	s.c. + i.p.	4	..	2	2
II	Subcutaneous tumor	F_2	s.c. + i.p. + i.v.	6	..	5	1
IIIa	Subcutaneous tumor	F_2	s.c. + i.p. + i.v.	3	2	..	1
	Subcutaneous tumor	F_2	s.c. + i.p.	4	..	2	2
IIIb	Ovarian tumor	F_2	s.c. + i.p.	4	1	1	2
	Ovarian tumor	F_1	s.c. + i.p.	3	..	3	..
IVa	Subcutaneous tumor	Af	s.c. + i.p.	4	..	..	4
	Subcutaneous tumor	F_2	s.c.	5	..	4	1
	Subcutaneous tumor	F_2	i.p.	4	..	4	..
IVb	Subcutaneous tumor	F_2	s.c.	7	1	3	3
Va	Fresh pooled tumor	F_2	s.c.	6	4	1	1
	Dried pooled tumor	F_2	s.c. + i.p.	8	..	..	8
Vb	Fresh pooled tumor	F_4	s.c.	6	..	..	6
	Fresh pooled tumor	F_2	s.c.	6	2	1	3
	Fresh pooled tumor	Ak	s.c.	4	2	2	..
	Dried pooled tumor	F_2	s.c. + i.p. + i.th.	4	..	..	4
	Dried pooled tumor	F_2	s.c. + i.p.	7	..	..	7
	Dried pooled tumor	F_1	s.c. + i.th.	4	..	..	4
VIa	Fresh pooled tumor	F_2	s.c.	3	1	1	1
	Frozen and thawed tumor	F_2	s.c. + i.p. + i.v.	5	..	2	3
VIb	Fresh pooled tumor	F_2	s.c.	3	1	1	1
	Frozen and thawed tumor	F_2	s.c. + i.p. + i.v.	5	1	3	1
VIc	Subcutaneous tumor	Ak	s.c.	5	1	3	1
	Subcutaneous tumor	Rf	s.c.	4	..	..	4
	Subcutaneous tumor	F_1	s.c.	4	1	3	..
VId	Subcutaneous tumor	Ak	s.c.	4	2	2	..
	Subcutaneous tumor	Rf	s.c.	5	..	..	5
	Subcutaneous tumor	F_1	s.c.	4	..	4	..
VIe	Subcutaneous tumor	Ak	s.c.	4	..	4	..
	Subcutaneous tumor	Rf	s.c.	5	..	..	5
	Subcutaneous tumor	F_1	s.c.	4	..	2	2
VIIa	Subcutaneous tumor	F_2	s.c.	5	1	..	4
VIIb	Subcutaneous tumor	F_1	s.c.	5	3	2	..
	Subcutaneous tumor	F_1	i.v.	5	5	..	..
	Subcutaneous tumor	F_1	i.ce.	5	..	5	..
	Subcutaneous tumor	F_1	i.p.	5	..	5	..
VIIc	Subcutaneous pooled tumor	Ak	s.c.	5	1	3	1
	Subcutaneous pooled tumor	Rf	s.c.	5	..	..	5
	Subcutaneous pooled tumor	F_1	s.c.	4	..	2	2
	Subcutaneous pooled tumor	F_2	s.c.	4	..	4	..
VIIIa	Subcutaneous tumor	Ak	s.c.	4	3	1	..
	Subcutaneous tumor	Ak	i.ce.	5	..	5	..
	Subcutaneous tumor	F_1	i.tr.	1	..	1	..
	Subcutaneous tumor	F_1	i.ca.	1	..	1	..
VIIIb	Subcutaneous tumor	Ak	s.c.	4	1	3	..
IXa	Subcutaneous tumor	Ak	s.c.	2	1	1	..
	Subcutaneous tumor	Ak	i.tr.	3	..	3	..
	Subcutaneous tumor	Ak	i.ce.	3	..	3	..
IXb	Subcutaneous tumor	Ak	i.tr.	2	..	2	..
	Subcutaneous tumor	Ak	i.ca.	3	..	3	..
IXc	Subcutaneous tumor	Ak	s.c.	4	..	..	4
Xa	Subcutaneous tumor	Ak	i.oc.	15	..	9	6
Xb	Subcutaneous tumor	Ak	s.c.	4	..	3	1
Xc	Subcutaneous tumor	F_1	s.c.	12	..	12	..
XI	Subcutaneous tumor, fresh	F_1	s.c.	4	..	4	..
	Subcutaneous tumor, frozen and thawed	Ak	s.c.	6	..	4	2
XII to XVI	Subcutaneous tumor, fresh	F_1	s.c.	19	1	18	..
	Subcutaneous tumor, fresh	Ak	s.c.	4	..	4	..

* Abbreviations. Routes of injections:
s.c. = subcutaneous
i.p. = intraperitoneal
i.v. = intravenous
i.ce. = intracerebral
i.th. = into the thoracic cavity
i.tr. = intratracheal
i.oc. = intraocular
i.ca. = into left heart

Location of transmitted tumors and metastases.—The tumors first grew at the site of inoculation and no distant metastases were found unless at least 24 days had elapsed between inoculation and death. Frequently the subcutaneous tumors rapidly penetrated through the abdominal wall and formed peritoneal implants with ascites. The peritoneal effusion was occasionally bloody. Similarly, there was often direct extension of the tumor into the thoracic cavity. Distant metastases were observed in the lungs and heart. Metastases occurred in the heart in 7 of 32 mice that lived 28 days or longer with subcutaneous tumors, while lung tumors occurred in 8. After intravenous inoculation tumors appeared in the lung, rarely in the myocardium, and in one case in the mediastinum.

Change in virulence.—The transfer from the spontaneous tumors was made into 4 F_2 mice which received 400 r of filtered x-rays preceding injection. When killed 131 and 135 days after inoculation, 2 of these mice had small subcutaneous tumors with no metastases, and 2 had no tumors. In the second passage all but 1 of the 6 inoculated mice had readily palpable tumors at the site of inoculation after from 20 to 30 days. In the third successive passage the incubation period has further decreased to from 7 to 30 days. This change of virulence in the course of the first three passages was accompanied by a change in the histological character of the tumor cells that will be described.

In subsequent passages the tumor had maintained its virulence except for one line. In one experiment in the ninth passage 4 Ak mice were injected subcutaneously with the usual technic but failed to develop tumors after 40 to 74 days. Three other Ak mice, injected 14 days later with material in the same line also failed to develop tumors after 29 to 61 days and thus this line of the strain died out.

Change in morphological character of strain A in the course of subpassages.—Spontaneous tumor (mouse Ma 387): This is ill-defined and is composed of two parts (Figs. 1 and 2). One is characterized by cuboidal or cylindrical cells that are hyperchromatic and vary greatly in size and shape. The second part, which merges into the former, is composed of cells that are not hyperchromatic, are in gland-like arrangement, and resemble very closely cuboidal alveolar epithelial cells seen in chronic diseases of the lung. About the region of contact of these two types of cells there are transitional forms. Indeed, there may be some doubt about the tumor-like character of the normally-staining gland-like structures but there is little if any doubt that the hyperchromatic part represents a new growth with cells exhibiting malignant characteristics. The differences in the size of the nuclei are marked; many cells have giant nuclei with relatively scanty cytoplasm.

In the course of the first subpassage this regularity of structure became lost. Glandular areas were still present but in many places the cells became elongated though not quite spindle-shaped and showed no particular arrangement.

Mouse Ma 389, first passage: The tumor in the subcutaneous tissue at the site of injection is circumscribed and is surrounded by masses of lymphocytes with strands of these cells extending into the tumor. There are numerous fibroblasts and a moderate number of collagenous fibrils in and about the tumor. In places this picture resembles that seen in regressing transmitted tumors. The appearance of the tumor cells varies in different parts of the section. In some places it resembles very closely the spontaneous tumor. There are a few elongated forms of tumor cells, but these are still in epithelial-like arrangement and are not definitely spindle-shaped. This tumor was not transmitted.

Strain A is derived from the subcutaneous tumor produced in mouse Ma 390, first passage (Figs. 3 and 4). Sections of this tumor show both cuboidal or polygonal cells in duct-like arrangement, spindle-shaped forms without duct formation and numerous transitional forms. There are also many duct-like spaces formed by elongated cells. There is a conspicuous absence of basement membranes in many places. The impression is gained that the absence of such a limiting membrane is intimately connected with the morphological transformation. The cells normally lining the ducts also grow outward and as they pass into the surrounding tissue assume spindle-shaped forms. Most cells which remain lining the ducts are polygonal; a

DESCRIPTION OF FIGURES 1 TO 7

Sections stained with hematoxylin and eosin; all magnifications × 340.

FIG. 1.—Photomicrograph of section from spontaneous lung tumor strain A (mouse Ma 387) showing the two types of cells, hyperchromatic and normally-staining.

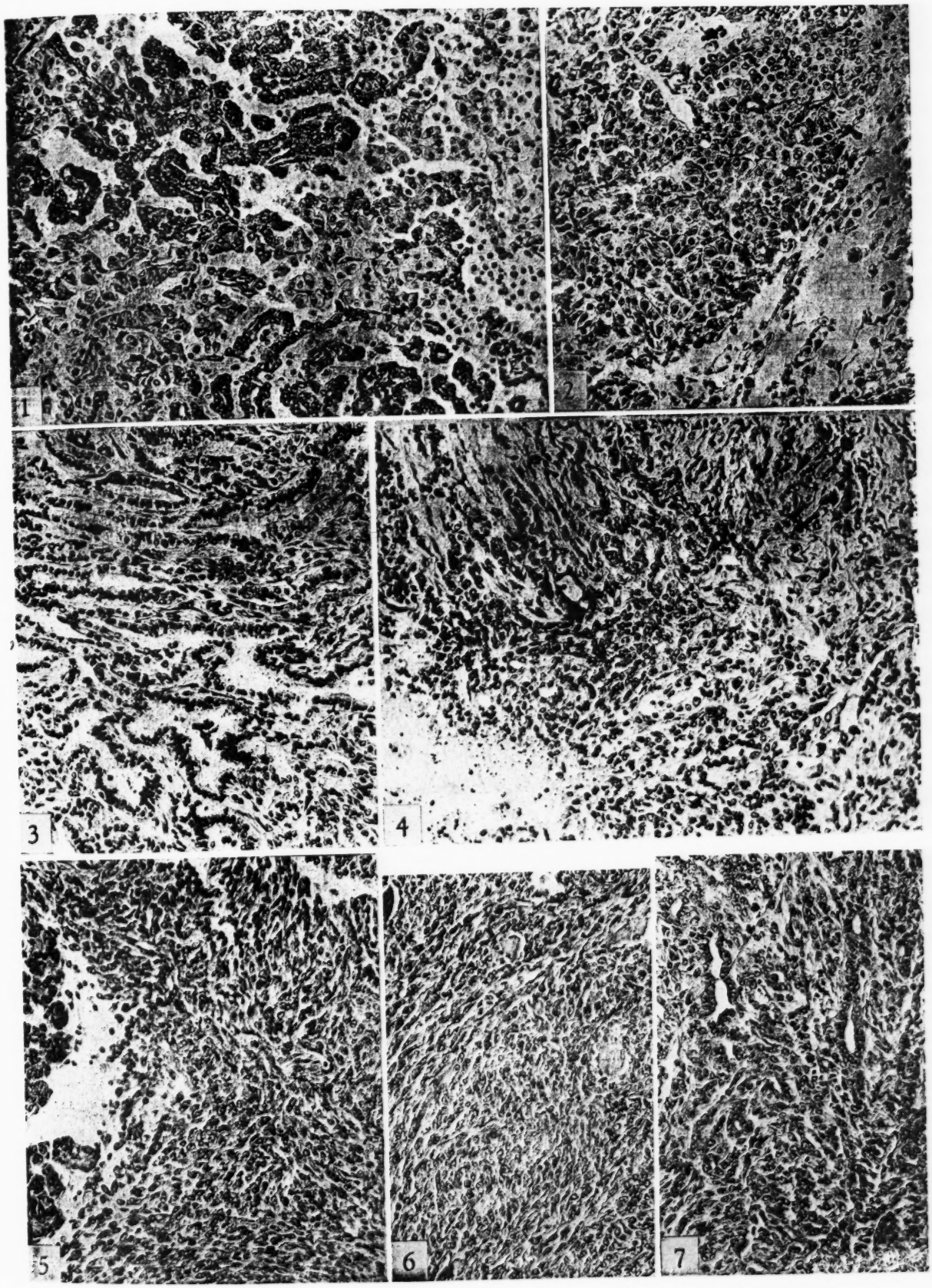
FIG. 2.—Section through the margin of the tumor.

FIGS. 3 and 4.—Sections from the subcutaneous growth of strain A lung tumors in mouse Ma 390 showing both cuboidal and spindle-shaped forms.

FIG. 5.—Section from second passage growth of lung tumor strain A produced by intraperitoneal injection in mouse Ma 552, showing spindle-shaped cells invading the pancreas.

FIG. 6.—Section of the second passage tumor in the subcutaneous tissue of mouse Ma 552, showing spindle-shaped cells invading muscle.

FIG. 7.—Section from the same subcutaneous tumor as Fig. 6, showing formation of ducts.



FIGS. 1 TO 7

few tend to be elongated; many bulge into the lumen of the ducts as narrow cylinders or as stout spindles. These lining cells are apparently restrained, whereas those growing outward proliferate with greater vigor as indicated by the number of mitotic figures. Transitional forms between the spindle-shaped cells and cuboidal epithelial cells are numerous as the tendency for gland formation becomes indistinct. A study of this section leads to the conclusion that the spindle-shaped sarcoma-like cells are derivatives of the cuboidal cells of the pulmonary adenoma.

Wells, Slye, and Holmes (8) explain this phenomenon as follows: "When the cancer cells get away from the alveolar walls which serve as a framework and perhaps maintain their epithelial character, they assume spindle-cell character." This explanation does not hold because our strain B, described below, and several of the tumors of Andervont, retained their epithelial character even in the subcutaneous tissue in numerous subpassages; that is, at a place distant from the alveolar walls.

In the second passage of strain A the cells are spindle-shaped and form whorls and bundles. There is only a suggestion of gland formation in few areas. The nuclei vary from round to elongated forms, are hyperchromatic and vesicular, and vary greatly in size. In all subsequent subcutaneous passages the spindle-shaped form is retained.

Section from the peritoneal tumor (mouse Ma 552, second passage, Fig. 5) shows only spindle-shaped tumor cells. In sections from the subcutaneous tumor (Figs. 6 and 7) there are solid epithelial-like and duct-like structures between areas of elongated sarcoma-like cells. The microscopic picture resembles very closely granulosa-cell tumors of the ovary which often contain duct-like structures and spindle-shaped mesenchymal cells with numerous transitional forms. The malignant character of this transmitted growth is evident. The nuclei of most cells are large, hyperchromatic, and the cytoplasm is relatively scanty. There is great variation in the size and shape of the nuclei and mitotic figures are numerous. There is a marked invasion of muscle tissue, and here, in addition to the discrete tumor cells, numerous syncytium-like masses of tumor cells are seen. Even in the duct-like areas these cells have evidently deviated considerably from those of the primary lung tumor.

In the preparation stained according to Foot's modification of Masson's technic the absence or scantiness of green-staining reticulum between tumor cells is noteworthy. The small amount of green-staining collagenous fibers, present in a few places, may represent preformed collagenous tissue. Of particular importance in this connection is the section from the peritoneal implants. In these, large masses of tumor cells are seen with almost no green-staining collagenous material. However, areas with dense growth of tumor cells with no green-staining intracellular material can also be found in known transmissible sarcomas; *e.g.*, Rous sarcoma, and this finding is therefore of little if any value in distinguishing spindle-shaped transmitted lung tumors from true sarcomas.

All lines of lung tumor strain A now carried are derived from the ovarian tumor of mouse Ma 557, (second passage) (Figs. 8 and 9). This arose after intraperitoneal inoculation and contains only spindle-shaped or polygonal forms such as those of the other peritoneal growths already described. There are large, clear spaces lined by elongated tumor cells. This section resembles the picture often described as endothelioma. It is noteworthy that this mouse was 109 days old when killed 53 days after intraperitoneal injection and that the ovarian tumor was the only evidence of a successful inoculation. Spontaneous ovarian tumors have not been described in young mice (5).

In sections from another mouse of the second passage (mouse Ma 556) spindle-shaped forms are predominant but definite duct-like areas are still present.

Fig. 1 (mouse Ma 834, seventh passage) shows a tumor in the lung produced by intravenous inoculation. This tumor contains both epithelial and sarcomatous areas indicating that a tendency to assume duct-like arrangement has not been entirely lost. About the bulk of the growth tumor cells are seen in the surrounding alveoli, detached or in small groups. Masses

DESCRIPTION OF FIGURES 8 TO 14

Sections stained with hematoxylin and eosin; all magnifications × 340.

FIGS. 8 AND 9.—Sections of the growth of lung tumor strain A in the ovary of mouse Ma 555, second passage, following intraperitoneal injection, showing spindle-shaped and polygonal cells.

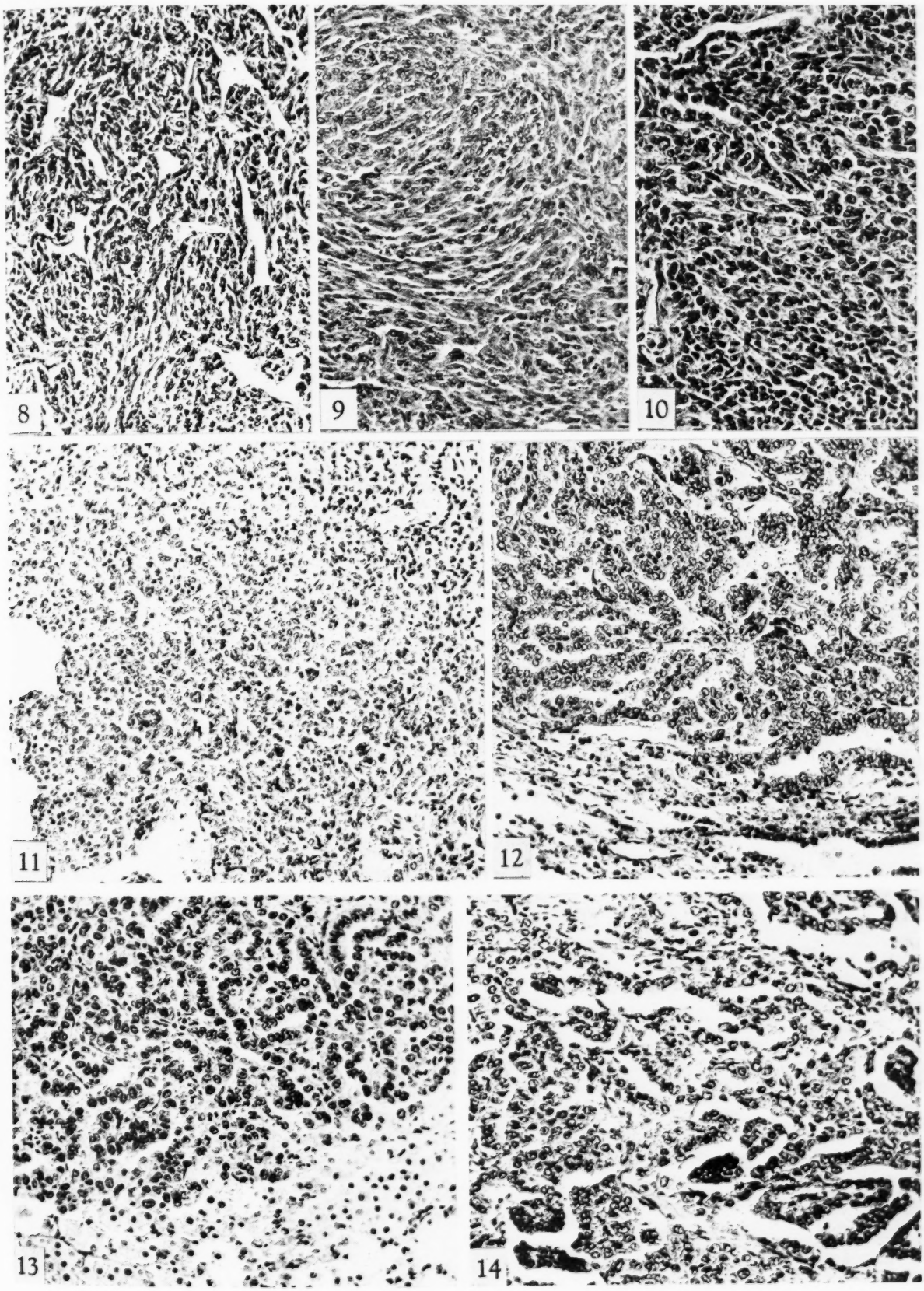
FIG. 10.—Section of the growth of lung tumor strain A in the lung of mouse Ma 834, seventh passage, produced by intravenous inoculation. This tumor contained both epithelial-like and sarcoma-like areas. Most of the cells in this photomicrograph are epithelial-like.

FIG. 11.—Section of original nodule of spontaneous lung tumor strain B, from lung of mouse Rfk 9, showing characteristic appearance of pulmonary adenoma.

FIG. 12.—Section of lung tumor strain B, first passage, in the lung of a mouse injected intravenously with a suspension of the original growth.

FIG. 13.—Section of lung tumor strain B, second passage, in the lung of a mouse following intravenous injection.

FIG. 14.—Section of lung tumor strain B, third passage, in subcutaneous tissue, showing an epithelial structure with slight tendency for the cells to assume spindle-shaped forms.



FIGS. 8 TO 14

of tumor cells (thrombi) are found in large branches of the pulmonary artery.

The number of tumors in the lung produced by intravenous inoculation or resulting from metastases was variable. In some cases only one or a few tumors were produced while in others they were almost innumerable.

Attempts to change the microscopic character of the tumor by growing it in different organs.—Tumor cells were introduced by different routes to ascertain whether the histological character of the tumor could be modified by environment. Such a modification was observed by Lucké (7) with transmissible adenocarcinoma of frog kidneys invading various structures of the eye.

The following routes of injection were used: subcutaneous, intraperitoneal, intravenous, intracerebral, intracardiac, intrathoracic, intratracheal, and intraocular. Growth resulted in the subcutaneous tissue and peritoneum including the peritoneal surface of the diaphragm, ovaries, kidneys, lungs, heart, pleura, mediastinum, brain, and eye.

The only place where the tumor cells showed gland-like formations was in the lung, after intravenous inoculation, but even here these structures were found in only a few places and were not conspicuous.

Table II: Transmission Experiments with Lung Tumor Strain B

Passage	Material injected	Stock of mice	Route of injection *	Number of mice injected	Number with lung tumor	Number with subcutaneous tumor	Number negative
I	Lung tumor (spontaneous)	Rf	s.c., i.p., i.v.	4	2	..	2
II	Lung tumor	Rf	s.c., i.p., i.v.	6	6	..	..
IIIa	Lung tumor	F_2	s.c., i.p., i.v.	4	..	..	4
	Lung tumor	F_4	s.c., i.p.	4	..	..	4
IIIb	Lung tumor	Rf	s.c., i.p., i.v.	5	5	..	..
	Frozen and thawed lung tumor	Rf	s.c., i.p., i.v.	5	..	..	5
IIIc	Lung tumor	Rf	s.c., i.p., i.v.	5	3	2	..
	Frozen and thawed lung tumor	Rf	s.c., i.p., i.v.	5	..	..	5
IVa	Lung tumor	Rf	i.v.	6	4	..	2
		Rf	i.cc.	7	..	..	7
IVb	Lung tumor	Rf	i.v.	8	6	..	2
			i.cc.	8	1	..	7

* Abbreviations explained in footnote to Table I.

Lung Tumor Strain B

Origin.—This strain originated in an Rf mouse (Rfk 9) that was killed at the age of 566 days. At autopsy, a spherical tumor, measuring 4 mm. in diameter, was found in the lung. In sections the tumor showed characteristic appearance of pulmonary adenoma with no evidence of malignancy. (Fig. 11).

Transmission experiments.—Four mice approximately 4 weeks of age of stock Rf were injected with particles from this spontaneous lung tumor by the combined subcutaneous, intraperitoneal, and intravenous routes. One of the injected mice died 136 days after injection and had a small nodule in the lung, presumably an early lung tumor. The remaining three mice were killed 184 days after injection; two appeared healthy but one had two small lung tumors, one of which is illustrated in Fig. 12. A second subpassage was made from the latter mouse in a similar manner. Six mice of stock Rf were injected when 105 days old; two were killed 90 days, three 99 days, and one 151 days after injection. None of these mice had subcutaneous tumors but all had numerous lung tumors, illustrated in Fig. 13.

The third successive passage was made from three different mice. The first group (passage IIIa) of recipient mice was composed of four F_2 and four F_4 hybrids, none of which had lung tumors when killed from 193 to 243 days after injection. The second group of mice (passage IIIb) were of the Rf stock; 5 of these received fresh and 5 frozen and thawed tumor particles. All of the former had lung tumors when killed from 93 to 215 days after injection but none of the latter, killed at 211 days after injection, had any neoplasm. Five mice of the third group (passage IIIc) received fresh and 5 frozen and thawed tumor particles. These mice were of the Rf stock and approximately 45 days old when injected. All of the mice receiving fresh tumor particles had lung tumors and 2 had also subcutaneous growths at the site of injection. One of these measured 6 mm. in diameter, the other 10 mm. Attempts are now being made to propagate this growth by the subcutaneous route, to determine the susceptibility of various stocks, and to diminish the incubation period of pre-irradiation with x-rays.

A fourth successive passage was attempted by intracerebral and intravenous routes. Of 8 mice injected intravenously (passage IIIa), 4 developed lung tumors. The 7 mice injected intracerebrally failed to develop tumors. In a second experiment (passage 3b), 6 of 8 mice injected intravenously developed lung tumors; of 8 mice injected intracerebrally one developed a lung tumor and none had tumors in the cranial cavity.

Character of lung tumor, strain B.—The properties of this transmissible tumor are of unusual interest. In the course of the first two passages, only the intravenous route of inoculation was successful and no tumors appeared at the site of subcutaneous or intraperitoneal injections. Had the usual routes been used for inoculation the results would have been negative and the inoculated animals regarded as resistant to this neoplasm. Even the brain, a soil generally believed to be very favorable for the growth of transmitted tumors, proved resistant to strain B.

The results of one series of inoculations in the third passage were likewise positive by the intravenous route only, whereas in another series 2 mice developed subcutaneous tumors although all 5 had lung tumors.

Tumors of strain B are unusually slow in growing. There are no exact data available on their incubation period but the tumors found in animals 3 to 6 months after injection were still minute. This observation emphasizes the necessity of keeping inoculated animals for longer periods of time in experiments aiming to establish resistance of the recipient animals to given neoplasms.

Because of the long incubation periods of these transmitted tumors, their localization in the lung, and their adenomatous character, evidence is wanted to indicate that these growths are not spontaneous. The tumors in the inoculated animals appeared at an earlier age and in greater percentage than spontaneous lung tumors of these stocks. Table II shows that in passages IIIc and IIId none of the mice injected with frozen tumor developed lung tumors although all mice injected with fresh tumors did so. All animals in these experiments were of the same age and stock at the time of inoculation and were killed at approximately the same age. Previous experiments have shown that many types of tumor cells are not destroyed by freezing. Moreover, the histological character of these tumors exhibits a slight but unmistakable difference from the usual type of spontaneous tumors. The latter are gland-like, sharply circumscribed with cuboidal or polygonal cells, and, in our stocks, very seldom exhibit evidence of malignancy. The transmitted tumors of strain B show, in addition to gland-like structures, elongated forms, numerous mitoses, and more definite invasion of surrounding structures.

Morphology.—A section from the spontaneous tumor (mouse Rfk 9, Fig. 11) shows a typical pulmonary adenoma. The cells are fairly uniform in size and shape and do not show hyperchromatophilia. Malignant-appearing areas, as in the spontaneous case from strain A, are not seen. The first passage tumor (Fig. 12) like that of the spontaneous adenomas is a sharply-circumscribed growth with a papillary adenomatous arrangement of cells not hyperchromatic.

In the second passage (Fig. 13), there is a slight deviation from the spontaneous pulmonary adenoma. More than one-half of the lung parenchyma is occupied by tumor that is ill-defined. There is no definite duct formation; papillary forms are certainly absent. Mitotic figures seem to be more numerous and there is a moderate degree of anaplasia. These tumors may already be regarded as adenocarcinomas. Alveoli about the tumor nodules contain many large mononuclear, presumably neoplastic cells but this is also seen in spontaneous tumors.

Fig. 14 shows the subcutaneous tumor of strain B (mouse Ma 838, third passage). The appearance of the tumor in the fourth passage (mouse Ma 900) differs from those previously described. It tends to grow more solidly though about one-half of it is still papillary and adenomatous. It compresses considerably the surrounding lung tissue in which many alveoli are completely atelectatic. There are also areas composed of cells which show a tendency to assume a spindle-shaped form and the impression is gained that this tumor is about to become spindle-shaped. There is a medium-sized artery about the tumor, the lumen of which is completely occluded with degenerating tumor cells. These tumor thrombi, not seen in spontaneous lung tumors, are evidence of the metastatic character of these growths.

DISCUSSION

Character of the lining cells of alveoli.—Neoplasms of the lung are at present often indiscriminately labeled as carcinoma, usually as bronchogenic in origin. The studies on the pathogenesis of the lung tumors of mice indicate that they arise as a rule from alveolar epithelium, though most human tumors are undoubtedly bronchogenic. The opinion that the alveolar lining cells in man cannot give rise to tumors is open to doubt because in chronic inflammation these cells often assume a cuboidal form and are capable of a mitotic division, and it is reasonable to suppose that they may undergo a malignant transformation.

It may be questioned, however, whether the tumors arising in the alveolar lining cells are properly labelled carcinomas. The histogenesis of the alveolar lining cells is fully discussed by Bargmann (3). According to the classical studies of Koelliker, which have been accepted by most histologists, the respiratory epithelium is of entodermal origin and is composed of two types of cells, a) polygonal, nucleated, measuring 7 to 15 μ in diameter, b) nonnucleated, thin plates of 22 to 45 μ in diameter. The plates originate from cuboidal cells after birth (3).

According to one of the contradictory views which found several adherents (3), the alveolar lining cells are mesenchymal, actively phagocytic, and therefore

related to the "reticulo-endothelial system." The studies on the histogenesis and transmissibility of lung tumor show them to represent a cell type different from the large mononuclear phagocytic cells of the spleen and liver.

The observations made with lung tumors of mice indicate that the alveolar lining cells have potentialities of assuming both epithelial-like and sarcoma-like forms. This behavior is similar to that of the serosal cells of the body cavities and of the mesenchymal cells of the ovary, which may resemble both epithelial and connective tissue cells.

The factors which determine the morphological appearance of these cells are not known. Wells, Slye, and Holmes (8) assumed that the framework of the alveoli was the determining factor, the lining cells assuming a spindle-shaped form in the absence of this framework. This view does not explain why certain lung tumors propagated in subpassages in the subcutaneous tissues retain a gland-like appearance. It seems that the transmissible lung tumors with gland-like appearance represent a more differentiated type of growth, whereas those composed of spindle-shaped cells represent a less differentiated type of greater malignancy.

The morphological studies lead us to conclude that the change in the character of the tumor is due to a modification in appearance of the tumor cells and not to an overgrowth of sarcomatous elements present in the original lung tumor. Such morphological studies are, however, not entirely conclusive. It is conceivable that a small amount of carcinogen transferred into the subcutaneous tissue with fragments of the lung tumor was responsible for the development of sarcoma which gradually replaced the glandular elements, but there is no evidence that lungs of mice with spontaneous tumors contain a carcinogen.

Affinity of strain A for heart muscle.—Affinity for heart muscle is characteristic of lung tumor strain A. This characteristic is noteworthy since the heart is known to be a rare site of metastatic tumors. We did not observe such an affinity in any of the numerous spontaneous and transmitted tumors of mice studied in this laboratory. Hematogenous metastases from this tumor are common in the lung yet other organs are rarely involved. This, along with numerous similar observations, points to the significance of special affinities varying with different tumors as one of the factors that determines the site of metastases.

SUMMARY AND CONCLUSIONS

Two transmissible tumors originating in adenoma of the lung of mice were studied. One (strain A) grew in the subcutaneous tissue, assumed a sarcoma-like appearance, and its growth rate became greatly enhanced.

Observations made suggest that this change is due to a modification in appearance and character of tumor cells in the course of transplantation and not to an overgrowth of sarcomatous elements present in the original lung tumor. This modification is fixed and is not altered by environmental conditions. Although the growth is sarcoma-like it produces few or no collagenous and reticulum fibers.

The second transmissible tumor (strain B) could at first be transmitted only by the intravenous route. It is characterized by affinity for lungs and by a long incubation period which has not changed in the course of four successive passages. In the course of successive passages it retained its adenoma-like character, unchanged by growth in the subcutaneous tissue.

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Studies in Malignant Tumors of the Testis

IV. Bilateral Testicular Cancer. Incidence, Nature, and Bearing upon Management of the Patient with a Single Testicular Cancer*

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In a previous communication we (7) suggested that testicular tumors, lymphosarcomas not included, possess a pronounced tendency to bilaterality. The man with cancer of one testis is exceptionally prone to development of cancer in the other testis. Thus, although the general incidence of testicular tumors among living males is approximately only 0.0013 per cent, the likelihood of involvement of the second testis is manifoldly greater, 0.7 per cent, in men who have had one testicular cancer. If the testes be ectopic the chances reach the grave values of 15 per cent with inguinal cryptorchidism, and 30 per cent with abdominal cryptorchidism. The tendency to bilateral tumors appears all the more pronounced in view of the fact that the majority of the patients live but a short time after the appearance of the first testicular tumor, and so afford limited opportunity for a later development of neoplasm in the other testis. Implications of these facts in the etiology of testicular cancer, and especially in the choice of management of the patient with cancer of the testis, have led to the following attempt to evaluate (a) the nature of the neoplasms which involve both testes of the same individual, (b) the possibilities of congenital predisposition to testicular cancer, and (c) certain basic facts in management.

Incidence.—Attempts at exhaustive examination of the literature from the time of Livingstone (17) in 1805 to La Manna (16) in 1939 resulted in the collection of 144 instances of bilateral testicular tumors, or 2 per cent of the approximately 7,000 total reported number of men with testicular tumors. That, despite the usually early death of individuals with testicular cancer, the incidence of bilateralism is as high as 2 per cent might seem on superficial appraisal to be ascribable to preferential reporting of the rather dramatic instances when both testes are cancerous. To test this possibility a survey was made of series from various hospitals in which only consecutive cases and not less than 10 patients were reported. The incidence of bilaterality in 1,466 such consecutive cases was 1.6 per cent, which serves to corroborate the high proportion of bilateralism in the other reports of testicular cancer.

The proportion of bilateral cancer is great in undescended testes; therefore the incidence of bilateralism will vary sharply, dependent upon the number of cryptorchid individuals included.

Pathological types.—In bilateral testicular cancer the relative percentages of the various types of tumors are similar to those which occur unilaterally save for the absence of chorionepitheliomas (Table I). The fact that no particular type of tumor is especially concerned in bilateral involvement is contrary to any hypothesis that bilateralism is due in large extent to metastases, for similar metastatic potentialities could scarcely be expected in dissimilar types of tumors.

Age distribution.—The distribution of cases according to the ages of the patients is shown in Fig 1. The highest peak of incidence falls in the fourth decade, being between 30 and 44 years in almost 40 per cent of all cases. In 116 cases, the average age of the patient when the first tumor was found was stated to be 40.6 years. It is noteworthy that a fourth of all cases with bilateral tumors were in men more than 50 years of age, a period of life at which unilateral cancer is much less frequent. A completely satisfactory explanation for this latter point is not obvious, but matters involved to some extent are the absence of bilateral chorionepitheliomas (a type restricted to the age of reproductive activity and infrequent in men over 40 years of age) and the relatively high incidence of bilateral teratomas during late years of life.

The youngest case was an 8-months' foetus with teratomas (23), the oldest a man 76 years of age who had unicellular cancer (3).

Figs. 2, 3, and 4 show that the increased incidence of testicular cancer during the years of reproductive activity applies to all types of tumors. The augmented occurrence of bilateral tumors during these years is due, however, chiefly to unicellular tumors, as shown in Fig. 5. Especial stimulation of unicellular types of

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TABLE I: COMPARISON OF BILATERAL AND UNILATERAL CASES OF TESTICULAR CANCER WITH REGARD TO PERCENTAGE OCCURRENCE OF VARIOUS TYPES IN DESCENDED AND IN RETAINED TESTES *

	Unicellular		Teratoid: Teratomas		Teratoid: Chorionepithelliomas		Malignant (type not known definitely)		Miscellaneous		Total	
Bilateral tumors	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Ectopic												
Inguinal	2	...	..	...	..	...	1	...	..	...	3	2.2
Abdominal	10	55.5	5	27.8	..	...	1	5.5	2	11.1	18	13.4
One inguinal, one abdominal	1	...	..	...	..	...	..	...	1	...	2	1.6
Scrotal	71	65.7	23	21.3	..	...	9	8.3	5	4.6	108	80.6
One ectopic, one scrotal	2	...	1	...	..	...	..	...	..	...	3	2.2
No data available	4	...	2	...	..	...	4	...	..	...	10	...
Total	90	62.5	31	21.5	..	...	15	10.4	8	5.5	144	...
Unilateral tumors												
Ectopic												
Inguinal	239	70.9	51	15.1	3	0.9	28	8.3	16	4.7	337	...
Abdominal	145	66.5	29	13.3	8	3.7	30	13.8	6	2.8	218	...
Scrotal	543	64.0	205	24.2	46	5.4	42	4.9	12	1.4	...	...
Total	927	66.1	285	20.2	57	4.1	100	7.1	34	2.4	1,403	...

* Figures for instances of bilateralism and ectopy represent attempts at complete culling of reported cases. A limited series of 848 cases of unilateral cancer in scrotal testes is included for purposes of comparison.

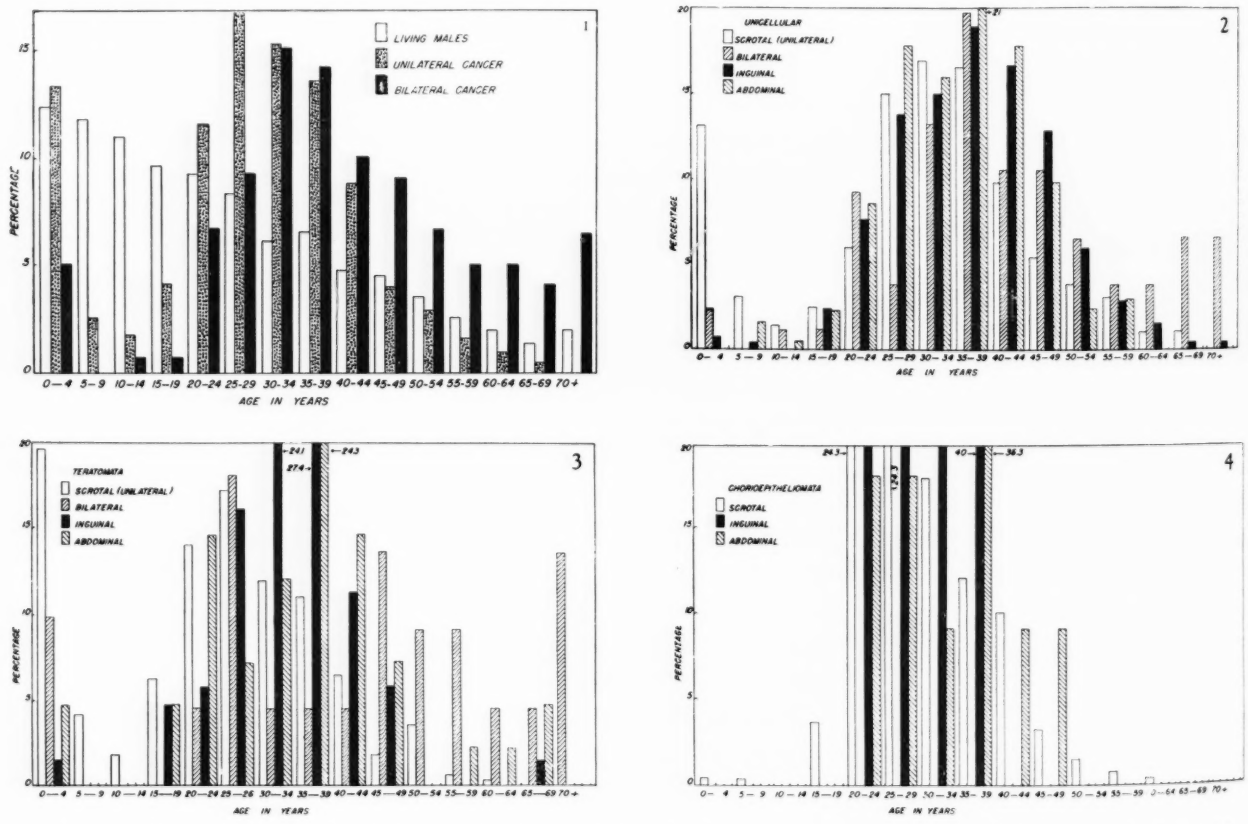

FIG. 1.—Comparison is made of the age distribution (when the first testicular cancer appeared) between 116 cases with bilateral cancer and 1,403 men with unilateral cancer. The average and the modal ages are greater in patients with bilateral testicular cancer than in those with a unilateral distribution of these tumors, and bear somewhat less of a relation to the years of reproductive activity.

FIGS. 2, 3, AND 4.—The age of the patients at the onset of the first tumor is compared for different types of bilateral cancer: unicellular (Fig. 2 of 1,090 cases) and teratoma (Fig. 3 of 434 cases). Comparison of these two types of tumor shows that the incidence is restricted more to the years of reproductive activity in unicellular tumors, although not as exclusively so as in chorionepithelioma (Fig. 4 of 85 cases). As neoplasms of chorionic tissue have never been observed bilaterally, the comparison represented in Fig. 4 is of unilateral cases only.

tumors, like stimulation of cells in the seminiferous tubules, might be expected during the years of reproductive life, but the preponderance of this type among bilateral tumors and the restricted period of years during which a man may develop chorionepitheliomas, are other noteworthy facts that need further examination with regard to the effect of endocrine substances.

Site of tumors.—The tumors were located in the scrotum in 108 instances, in the inguinal region in 3, in the abdomen in 18, and in a mixed distribution in 5 cases (Table I). The location was not stated in 12 reports. The frequency of bilateralism in abdominal testes is striking. Although the ratio of the abdominally-retained to the inguinally-retained testes (uncomplicated by cancer) is only 1 to 8, the ratio of occurrence in bilateral cancer is 6 to 1, a preferential incidence nearly 50 times greater than expected by chance association. This supports the previous suggestion of Gilbert and Hamilton (7) that the propensity for carcinogenesis is greatest in abdominally-retained testes. In the previous report in which unilateral and bilateral cancer had been considered jointly, the frequency of cancer in abdominal testes was about six times greater than expected from the ratio of the incidence of uncomplicated cryptorchidism in the abdomen and inguen.

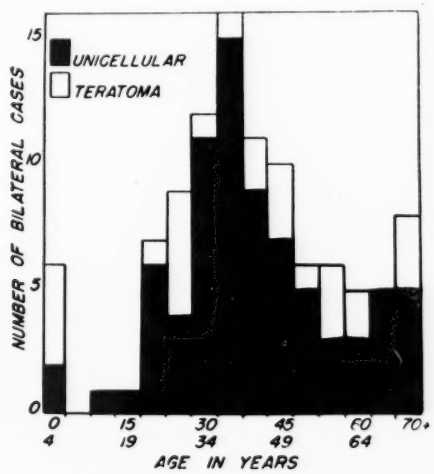

FIG. 5.—The age distribution of 103 men when they developed the first of 2 testicular tumors, shows that unicellular tumors, which form the majority of instances of testicular cancer, are the types concerned in the greatly augmented incidence of testicular cancer during the years of reproductive activity.

Etiology of testicular cancer.—As shown previously, the correlation between maldescent and cancer is high, more than 1 in every 10 instances of cancer being in an improperly descended testis, an association held to be about 48 times that expected as coincidental. No proof was found, however, that residence in ectopy *per se* rendered the testis potentially malignant. On the basis of these facts, the logical queries concern (a) the nature of the influences causing the maldescent and congenital defects, and (b) the possibility that those influences may predispose the testis to later malignant growths.

A survey of data concerning bilateral testicular tumors in comparison with testicular cancer in general suggests that prenatal factors are of even greater significance in bilateral than in unilateral cancer. Thus, 1 in every 5 instances of bilateral cancer occurs in congenitally undescended testes, an exceedingly high figure in view of the fact that the incidence of cryptorchidism is only 0.23 per cent and bilateral cryptorchidism only a small fraction of this. Moreover, 12.5 per cent of all bilateral cancer is in abdominally-retained testes, an exceedingly rare type of retention which represents the more severe form of maldescent. It is noteworthy that 1 of every 8 instances of bilateral testicular cancer occurs in the probably less than 0.005 per cent of men who have bilateral abdominal cryptorchidism.

Even though the number of cases is small, it is noteworthy that the percentage incidence of teratomas is twice as high in bilateral as in unilateral testicular cancer. This is interpreted as a further indication of a relationship between congenital factors and bilateral testicular cancer.

Several facts suggest that an abnormal influence by steroid substances, such as estrogens for example, is associated with cryptorchidism. In rodents according to Burrill, Greene, and Ivy (5), and in opossums as shown by Burns (4) prenatal treatment with estrogens prevents proper testicular descent. In man cryptorchidism is sometimes accompanied by hypospadias, an effect known to be produced in young female and male rats by estrogens, as described by Hain (10) and by Greene, Burrill, and Ivy (8).

Testicular cancer appears chiefly during the years of reproductive activity, suggesting that the endocrine stimuli existent during this period of life may be implicated also in the carcinogenesis. Any immediate conclusion that gonadotropic substances are directly and solely responsible, however, cannot be accepted, for androgens and some other substances stimulate at least the seminiferous tubules. Nevertheless, testicular cancer bears a high correlation with cryptorchidism, and at least in animals bilateral cryptorchidism is characterized by excessive secretion of gonadotropins. It is perhaps more than coincidental that testicular tumors (a) occur mainly during the years after gonadotropins have begun to be secreted in the greatest quantity and (b) are characteristically frequent in the cryptorchid state which has a tendency at least in animals to allow over-production of gonadotropins. Moreover, the maldescended testis resides in an environment where in rodents it is known to function ab-

normally and to react peculiarly to androgens and to gonadotropins as shown by the investigations of Hamilton and Leonard (12) and Mimpriss (20). Finally, cancer occurs only infrequently, if at all, in eunuchoid men whose testes are stimulated by only small quantities of gonadotropic substances.

The significantly lesser frequency of carcinogenesis in inguinal than in abdominal cryptorchidism contradicts the claims that traumatic forces play a large role in the induction of cancer in the ectopic testis. Protection provided by the abdomen would be expected to cushion the blows and prevent much of the stress from muscular movement of the abdominal wall.

Recognition and pre-operative duration.—The average pre-operative interval between the first tumor and removal of one or both testes was 16 months. The duration of the condition prior to consultation was, on the right side, 14.4 months, on the left, 10.3 months.

Clinical grades.—There were 82 cases which, when observed at the appearance of the first tumor, were classified in group I (operable cases stated to have no metastases) and underwent orchidectomy. It is eminently significant that 66 of these had bilateral orchidectomy with the findings of a usually unsuspected tumor in the second testis. In group II, consisting of 26 cases which were operated upon despite metastases, 21 of the men were orchidectomized bilaterally, while 5 had only unilateral orchidectomy performed.

Pathological types.—Definite histological data are available in 126 cases, excluding 11 tumors classified only as clinically malignant. There were 88 unicellular tumors, 30 teratomas, and 8 miscellaneous tumors, the last-named group including 4 adenomas, 2 interstitial cell tumors (in brothers) (14), one classified as adrenal carcinoma (24), and one as spindle cell sarcoma (15). A summary of data from the case records of these types of tumors follows.

I. Unicellular tumors.[1]—There were 88 such tumors. The average age of 76 patients was 41.8 years. The site was stated to be as follows: scrotal, 71; inguinal, 2; and abdominal, 10. Mixed distribution was scrotal and abdominal in two men, abdominal and inguinal in one man. This group includes one pseudohermaphrodite described by Christophe and coworkers (6).

The average pre-operative duration, as stated in reports of 60 cases, was 18.2 months; in 34 instances where the right side was involved, the average was 16.5 months, whereas with 24 tumors on the left side, it was 12.5 months. The average post-operative duration in cases terminating fatally, as stated in 40 instances, was 18.2 months. The average total length of life from the onset of the condition until death, as stated in 29 cases, was 29.7 months. Thirty-two patients survived for an average of approximately 4 years (47.6 months) before death or development of a second tumor; of these men, 25 were stated to be alive and well after a mean interval of 58.7 months.

[1] Variation among different authors in the classification of tumors renders unwise the separation of unicellular tumors according to more distinct histological types.

The second tumor was recognized in 18 instances at an average of 55.5 months after removal of the first tumor. Of these, 5 patients died at an average of 4 years after the first operation, the longest survival before development of the second tumor being 12 years (21). The remaining 13 patients survived an average of 5 years before the second tumor developed, but no further follow-up is available; the longest survivals before the second tumor appeared in this group were 10, 12, 14, and 23 years.

There were 60 patients in group I, of which 46 had bilateral, 4 right- and 4 left-sided, orchidectomy. Group II comprised 12 patients, of whom 10 had bilateral, and one each right or left orchidectomy. Sixteen patients with bilateral tumors were considered inoperable, and no surgery was attempted.

II. Teratoid tumors.—In 30 cases with teratomas microscopic examination (not always serial) revealed no evidence of choriogenic components. The average age of the patient, stated in 25 instances, was 39.1 years. The pre-operative duration for 20 cases was 8.6 months. The length of life when the right testis was first involved averaged 10 months in 13 instances, 6 months in 11 cases when the first site was the left testis. The post-operative duration of 14 fatal cases averaged 24.4 months, the total duration 32.0 months. Fourteen patients were reported alive at an average of 84.3 months, 9 of these survived 9.5 years, while 5 were alive with metastases or a second tumor, after an average of 28.6 months. Six patients survived 5 years or more, with an average of 140 months (12 years); 4 of these were alive at an average of 144 months (12 years), one with metastases at 8 years. Two died later, due to involvement of the second testis, 10 and 13 years respectively after an initial orchidectomy (22, 11).

In 9 cases the second tumor developed at an average of 8 years after unilateral orchidectomy; 6 of these men remained alive at an average of 86 months before the second tumor developed. Six patients developed the second tumor from 2 to 60 months after orchidectomy.

There were 13 patients in group I, 12 undergoing bilateral and 1 right orchidectomy. Group II comprised 11 patients, 9 with bilateral and 2 with left-sided orchidectomy. Group III contained 6 patients, 2 being pseudohermaphrodites (1, 2, 9).

III. Malignant testicular tumors.—Eleven tumors were classified as clinically malignant without specific pathology even in cases where autopsies were done. One patient (25) developed the second tumor 72 months after removal of the first testis, but no further follow-up is available. One pseudohermaphrodite is included (19).

IV. Miscellaneous tumors.—Seven individuals with these tumors underwent bilateral orchidectomy. Five were in group I, and one in group II; only one had no surgery performed (13). No significant clinical data are given, except that in one instance of an interstitial cell tumor the second testis became involved 3 years after removal of the first testis (14). This patient's brother also had bilateral orchidectomy because of an interstitial cell tumor. The case reported by Hermanns, without operative notes at the time, had castration phenomena, gynecomastia, and indurated atrophic testes which were described as adenomatous. Two pseudohermaphrodites (18, 26) had adenomas.

SUMMARY AND CONCLUSIONS

In men with one testicular cancer, the likelihood of cancer in the second testis is from several hundred to several thousand times greater than expected in chance association.

Abdominally-retained testes are especially prone to bilateral cancer. One of every 8 men with bilateral

testicular cancer had abdominally-retained testes, a condition which can be estimated to occur in probably much less than 0.005 per cent of men. The pronounced carcinogenic tendency of abdominal testes can also be ascertained differently. Although the incidence of cancer in inguinal testes is high, the occurrence in abdominal testes is some 50 times higher, as seen by comparison with the ratio of abdominal and inguinal testes in uncomplicated cryptorchidism.

Bilateral tumors occur predominantly in the reproductive years of life, but the first tumor usually occurs later in life than in unilateral cases. The greatest number of both unilateral and bilateral tumors, especially those occurring during the reproductive years, are unicellular types.

The relative percentages of the various types of tumors are the same in bilateral as in unilateral cancer, save for the fact that chorionepitheliomas are not found bilaterally.

Further evidence is advanced to the effect that prenatal factors are significant in the predisposition to testicular cancer. It is possible that estrogens might play a role in the instigation of cryptorchidism and gonadotropins in the stimulation to carcinogenesis during the reproductive years of life.

Management of the case with cancer of one testis must be concerned with the possibility of primary involvement of the other testis. Despite the usual rapidly fatal course in the patient with testicular cancer, a tumor has appeared in the other testis in 15 per cent of those with inguinal testes and 30 per cent of those with abdominal testes. The second testis of a man with a bilateral cryptorchidism and cancer of one testis must be viewed as a site of a potential carcinogenesis.

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Radioactive Phosphorus as a Therapeutic Agent in Malignant Neoplastic Disease*†

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Radioactive phosphorus, or P^{32}, as a therapeutic agent in malignant disease, has been studied at the Memorial Hospital since January, 1940. Investigations have been carried on by a large group of workers in several clinical and laboratory departments. Their individual investigations are to be published separately. It seems desirable however, to present a summary of the principal findings in the various problems investigated to bring out the inter-relations of the different aspects of the work, and to indicate further desirable extensions. The author, who has been associated with all parts of the study, was requested to prepare the present paper.

Initially, the investigation followed the original work of Lawrence and associates (2, 4, 7, 8, 9, 11) and was limited to patients with leukemia. It was found, as Lawrence had previously stated, that the leukemic cells and the tissues which were infiltrated with them absorbed greater amounts of radioactive phosphorus than did normal leukocytes or noninfiltrated tissue. The study was then directed toward other types of malignant neoplasms in the hope that a similar selective absorption might be found.

PREPARATION AND GENERAL PROPERTIES OF RADIOACTIVE PHOSPHORUS

Radioactive phosphorus, P^{32}, is produced by bombarding red phosphorus with deuterons ($_1H^2$) in the cyclotron. The following reaction takes place:

$$_{15}P^{31} + {}_1H^2 \text{ (deuteron)} \rightarrow {}_{15}P^{32} + {}_1H^1 \text{ (proton)}$$

The radioactive red phosphorus [1] is converted to disodium acid phosphate, Na_2HPO_4, by suitable chemical means, and in this form it can be administered either orally or parenterally. As used at present, the phosphate is in an aqueous solution containing 15 mgm. of phosphate per cc., with an activity of about 300 microcuries (μc.)[2] per cc. of solution.

Our average single therapeutic dose for a 70 kg. adult varies between 1.5 and 4.0 mc. Therefore, between 5 and 15 cc. are administered at a time, and the total amount of phosphate in a dose varies between 75 and 225 mgm. It is estimated that about one in ten million of the phosphorus atoms in this solution is radioactive. The actual amount of radioactive phosphorus administered is thus extremely minute, between 0.00005 and 0.0001 mgm. for a single dose.

This isotope, P^{32}, has an unstable nucleus whose half life is 14.3 days and disintegrates according to the following formula:

$$_{15}P^{32} \rightarrow {}_{16}S^{32} + \beta \text{ (beta particle)}$$

The beta particles produced by the disintegration have an average energy of 700 kv. and can penetrate several meters of air, or between 2 and 4 mm. of tissue. This particle has about the same average energy as the beta particle ejected in soft tissue by 2,000,000 volt x-rays.

The unit of measurement of the radioactivity of P^{32} is the international millicurie (mc.) which, for any radioactive element, is the quantity in which occur 3.7×10^7 distintegrations per second. Thus, a millicurie of radioactive phosphorus, a milligram of radium *per se*, or a millicurie of radon *per se* are identical insofar as the number of atomic disintegrations per second are concerned. They differ vastly, however, in that radium and radon emit alpha particles, and radioactive phosphorus emits only beta particles. Also, in a milligram of radium in equilibrium with its products there is present one millicurie of each of the other members of the series, each disintegrating at the same rate of 3.7×10^7 atoms per second and emitting its own radiation. The beta and gamma radiations attributable to radium and radon come from the radium B and radium C in this equilibrium. It cannot be said, therefore, that one milligram of radium or one millicurie of radon and one millicurie of radioactive phosphorus will produce

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[1] The author is indebted to the Crocker Radiation Laboratory of the University of California for the radioactive phosphorus used in these investigations.

[2] 1 microcurie (μc.) equals 0.001 millicurie.

identical biological effects when used in the same fashion, nor can it be said that they produce identical amounts of radiation.

If the concentration of the radioactive phosphorus in a tissue is known, it is possible to estimate the radiation dose delivered to that tissue by the isotope. Through suitable formulae which take into account the energy of the beta ray, the number of disintegrations per second, and the half life, this dose can be expressed on an ionization basis to correspond closely to doses delivered with x-rays. For convenience, doses thus determined are expressed in "roentgen equivalents" (10). For example:

1. If 1.1 μc. of P^{32} remain in a kg. of tissue until it has completely disintegrated, 1 "roentgen equivalent" of radiation will be delivered to that tissue.

2. If 1 μc. of P^{32} remains in a gm. of tissue for 24 hours, 43 roentgen equivalents will be delivered to that tissue.

3. If 1 mc. is retained for 24 hours by an adult of 70 kg. weight, 0.6 roentgen equivalents of whole body radiation are delivered.

Treatment with radioactive phosphorus is simply another form of radiation therapy. Its single significant difference from x-ray or radium therapy is that it is administered orally or parenterally and distributed throughout the whole body, and the sources of the radiation are all within the tissues where the material is present. Its localization in any tissue is, so far as is known, purely a metabolic phenomenon and takes place in accordance with the metabolic needs of the various tissues for phosphorus. Therapy with radioactive phosphorus is, therefore, systemic irradiation. Hence, in the treatment of any malignant neoplasm, its effectiveness will depend on its distribution within the body. If it should prove to be more effective than conventional radiation therapy, it will be so because of this, and especially if there is a favorable differential absorption by scattered tumor cells.

TRACER STUDIES

If a small amount of radioactive material is administered to a living organism, subsequent ionization measurements can show how this substance is distributed among the various tissues (4, 9). Such tracer studies are useful for the investigation of various problems in metabolism; they also furnish a guide to possible therapeutic values, for it is believed that the findings after tracer doses probably indicate the differential absorption that would occur after therapeutic doses provided that the difference between the amount of phosphate in the tracer dose and the therapeutic dose is not large. Hence, if neoplastic tissues absorb amounts sufficiently greater than normal ones, the material should be a useful clinical adjunct. Such studies have been carried out for P^{32}. This work will be reported in detail elsewhere and will only be summarized briefly in this article (6).

In these studies, small amounts of radioactive phosphorus, about 30 per cent of our usual single therapeutic dose, were administered to a group of patients with various malignant neoplasms, at varying times before operation. Patients with the following conditions were selected for this investigation: carcinoma of the breast, osteogenic sarcoma, and lymphosarcoma. Portions of the different tissues in the surgically excised specimens of these patients were weighed, ashed, and their radioactivity measured and corrected for decay to the date of administration of the P^{32}.

In order that an adequate comparison could be made of the absorption by neoplastic tissue with that of normal tissue, whether in the same patient or in different patients, it was necessary to devise a suitable method. This was done by determining in every instance the ratio of μc. of P^{32} measured per kg. of tissue to the μc. of P^{32} administered per kg. of body weight.

All the calculations were based upon the amount *administered* per kg. of body weight. This figure indicates how much of the isotope would be present in any tissue if it were equally distributed throughout the body. If this quantity is divided into the amount actually found to be present in 1 kg. of any tissue, there is established the ratio between the amount of phosphorus actually taken up by that tissue and what it would have contained if the isotope had been equally distributed.[3] This ratio of differential absorption indicates approximately how much of the isotope will reach different tissues after a measured amount of the P^{32} is administered, provided that the amount of phosphate in the dose is not large. The ratio also shows at once whether a neoplastic tissue absorbs an amount of the isotope sufficiently above the average for the entire body to make it a probably useful method of therapy. If, for example, a neoplastic tissue has a differential absorption ratio of 1, it will receive no more radiation from radioactive phosphorus than will the whole body. On the other hand, if the ratio were 6, it would receive 6 times as much radiation as the average body tissue. In the first instance, P^{32} would be expected to be of little value, and in the second instance, it could be of significant therapeutic effect.

[3] For example: If an adult of 70 kg. is given 1 mc. (1,000 μc.) of P^{32}, 14.3 μc. (1,000/70) per kg. of body weight is the administered dose, corrected for decay to date of administration of the isotope. If, then, in this same adult, a cancer tissue absorbs 28.6 μc. per kg., the 28.6 is divided by 14.3. The resultant figure of 2 is the differential absorption ratio for that cancer tissue.

The ratio has been based on the administered dose rather than on the retained dose because in many instances it was not possible to measure the excretion of the isotope and thus determine the actual value of the latter. This method is satisfactory, since all figures would change proportionately if the retained dose instead of the administered dose were the basis of the ratio. Admittedly, the ratio may be different on different days for the same tissue in the same patient. However, this can be compensated for by making observations on a large group of patients at different intervals after the administration of a tracer dose of P^{32}. Such a series of observations will show if the isotope is retained a sufficient length of time to deliver its effective radiation, a matter of 3 weeks.

This ratio can probably be decreased or increased to some extent by varying the total amount of phosphorus administered. In all of the tracer studies, however, it did not exceed 10 per cent of the average patient's daily intake of 1 gm. of phosphorus.

Table I: Differential Absorption Ratio of Radioactive Phosphorus for Patients with Carcinoma of the Breast

Case number	μc. P^{32} administered per kg. of body weight	Days between administration of P^{32} and operation	Differential absorption ratio							
			Primary tumor	Breast tissue	Metastatic node	Normal node	Muscle	Fat	Skin	Blood
44551	5.6	1	1.3	0.2	..	1.8	0.7	0.2	0.3	0.8
59863	4.9	2	0.8	0.4	..	2.4	0.8	0.1	0.3	0.7
59714	2.6	5	1.9	0.3	3.3	2.5	1.2	0.2	0.5	0.5
59935	5.4	5	0.8	0.2	..	1.3	0.9	0.1	0.2	0.3
59995	9.1	5	..	..	4.0	2.8	1.3	0.1	0.4	0.4
59998	5.5	5	1.6	0.3	3.6	1.6	1.0	0.1	0.5	0.1
59953	3.0	7	1.0	0.2	1.9	1.6	1.0	0.1	0.2	0.5

Carcinoma of the Breast

This lesion was selected for study primarily because its frequency made available a sufficient number of cases in a relatively short time. A further reason was that the figures obtained from the study of this type of neoplasm should indicate to some degree what absorption of the phosphorus would take place in other types of carcinoma.

Table I shows the results of the analyses of tissue from patients with breast carcinoma. The differential absorption ratio for the primary tumor varied between 0.8 and 1.9. There is, therefore, little reason to expect that radioactive phosphorus could be used for primary therapy in this disease. A dose of 500 μc. per kg. of body weight would administer only between 450 and 1,000 "r" to the primary tumor. This dose of the isotope is about as great as can be safely administered to an adult of 70 kg. weight and would probably provide about 300 "r" whole body radiation during the period of its administration, a matter of about 12 or 14 weeks.

The lymph nodes which contained metastatic foci of breast carcinoma had an absorption ratio of from 1.9 to 4.0. Hence, the dose just discussed would deliver to the nodes between 1,000 and 2,000 "r" tumor dose. The higher absorption by the metastatic areas suggests that the radioactive phosphorus might be a useful therapeutic adjunct in patients who are to be treated by x-ray. This is on the grounds that the administration of the phosphorus might add sufficient supplementary radiation to that delivered by x-ray treatments to inactivate the disease. Because the radioactive phosphorus probably is handled as a normal metabolite, it is to be expected that it would reach all the metastatic foci outside the area treated by x-rays. It is thus possible that a sufficient amount of the isotope might be absorbed by the minute subclinical foci of metastatic cancer to inactivate or destroy them.

Osteogenic Sarcoma

Phosphorus is a normal constituent of bone, whose metabolism is in a large degree controlled by an alkaline phosphatase (13). Many osteogenic sarcomas are rich in it (13), a fact which is often reflected by a high serum phosphatase. The phosphatase in the tumor increases the need of that tissue for phosphorus. It seemed quite likely, therefore, that radioactive phosphorus would be absorbed by osteogenic sarcoma in sufficiently large quantities to make it a useful therapeutic agent. It also seemed possible that the minute metastatic foci might absorb sufficient phosphorus to be destroyed. If this last should prove to be true, then radioactive phosphorus would offer a real therapeutic hope in a disease which is fatal in about 90 per cent of the patients within two years from the time of diagnosis.

Table II shows the results of the analyses of tissues from patients with osteogenic sarcoma. The primary

tumor showed a differential absorption ratio that varied from 0.6 to 8.3. Those regions with the lowest absorption ratio were regions in which the tumor was hemorrhagic or had otherwise degenerated. The areas most actively growing were the ones with the highest absorption ratios. If we use the figure 4 as an over-all average absorption ratio for the primary tumor, for a dose of 500 μc. per kg. of body weight, it would receive about 2,000 roentgen equivalents tumor dose, too little to provide a primary method of therapy. However, if the only method of therapy to be used is x-radiation, the administration of 500 μc. per kg. of weight of the isotope would provide the advantage of at least a 30 per cent increase in the usual roentgen tumor dose which, in osteogenic sarcoma, does not usually exceed 6,000 to 7,000 r, and is limited by the tolerance of the skin for radiation. Skin damage is not a factor in therapy with radioactive phosphorus; rather, as will be shown later, the limiting factor is probably damage to bone marrow.

In only one case are measurements of the amount of P^{32} absorbed by metastatic tumor tissue available. In this instance, the ratio varied from 1 to 5, a fact which indicates that the radioactive phosphorus does reach distant metastases and might provide sufficient irradiation to inactivate them. Minute foci of metastatic disease are probably not too securely established. It is conceivable then that if they were damaged, their destruction might be completed by some bodily defense mechanism. Frequently, metastases appear months or years after the primary tumor has been destroyed. Hence, it may be supposed that they were present before the destruction of the primary tumor. Some force has kept them in the quiescent state for a period and, if to this force further damage could be added, it is not impossible that total destruction might result.

Table II: Differential Absorption Ratio of Radioactive Phosphorus in Patients with Osteogenic Sarcoma

Case number	μc. P^{32} administered per kg. of body weight	Days P^{32} administered before operation	Differential absorption ratio							Remarks
			Primary tumor	Primary tumor	Primary tumor	Femur	Tibia	Muscle	Epiphysis	
62057	7.3	1	5.8	1.7	1.1	0.7	0.6	0.7	..	Heavy irradiation 6 months before operation
61190	9.7	2	4.6	1.0	0.6	0.4	..	0.7	..	No preoperation irradiation
59700	2.2	3	3.1	..	..	..	0.8	1.9	1.5	No preoperation irradiation
59044	7.6	5	8.3	4.0	2.4	1.7	..	0.9	..	Heavy irradiation 2 weeks preoperation
59202	41.0	7	2.6	..	..	0.4	..	0.2	0.7	Heavy irradiation 2 weeks preoperation

Lymphosarcoma

The relative radiosensitivity of this type of lymphoma made it seem likely that this disease could be effectively treated with radioactive phosphorus.

Table III shows the results of the analyses of lymphosarcoma tissue. Because only local biopsy was done, no normal tissue was available to serve as a control. The differential absorption ratio varied between 1.6 and 12.0, with an average of about 3. It is to be noted that there are varying lengths of time between the administration of the isotope and the biopsy, but that the ratio remains as nearly constant as can be expected. This suggests that, once the radioactive phosphorus has been absorbed by the lymph node, it tends to remain in it for at least 2 weeks.

If a total dose of 500 μc. per kg. of body weight were administered to one of these patients, the diseased nodes would, on an average, receive about 1,500 roentgen equivalents tumor dose. This is in excess of the usual x-ray dose and suggests that radioactive phosphorus should be a useful method of therapy in

Table III: Differential Absorption Ratio of Radioactive Phosphorus in Patients with Lymphosarcoma

Case number	μc. of P^{32} administered per kg. of body weight	Days between administration of P^{32} and biopsy	Differential absorption ratio	
			Lymph node	Blood
SED	1.7	5	4.2	0.8
ORI	9.6	5	3.1	0.5
ANG	5.5	6	2.4	0.3
ABB	4.7	8	3.1	0.3
SEA	13.3	11	2.2	..
CLE	12.6	13	4.5	0.3
MAR	9.2	19	1.6	0.2
MEG	7.0	31	12.0	0.05

lymphosarcoma. The isotope would reach all lymph nodes and conceivably could destroy or inactivate all areas of disease.

Normal Tissues

Sufficient data have not yet been collected from patients coming to autopsy to permit accurate estimation of the amount of the isotope that will be absorbed by normal liver, spleen, lymph nodes, or kidney.

The relative radiosensitivity of the various normal organs as contrasted with malignant neoplastic tissue is also of importance. Normal liver and kidney are not radiosensitive, but are actually radioresistant. If their ratio does not exceed 1.0 to 1.5, they would probably be able to absorb the amount of radiation they would receive during a therapeutic trial of the isotope in such a disease as osteogenic sarcoma without, in all probability, showing radiation damage.

Therapeutic Studies

The proper method for the therapeutic administration of radioactive phosphorus is still unsettled. Originally, large single doses were administered with the amount only roughly related to the weight of the patient. These doses were repeated at approximately weekly intervals until, in leukemia, satisfactory reduction of the leukocyte count occurred. More recently, dosage has been related directly to body weight. The disease to be treated, the stage of the disease, and the patient's general condition are then carefully evaluated and become the deciding factors in the control of initial dosage. Patients in good general condition and

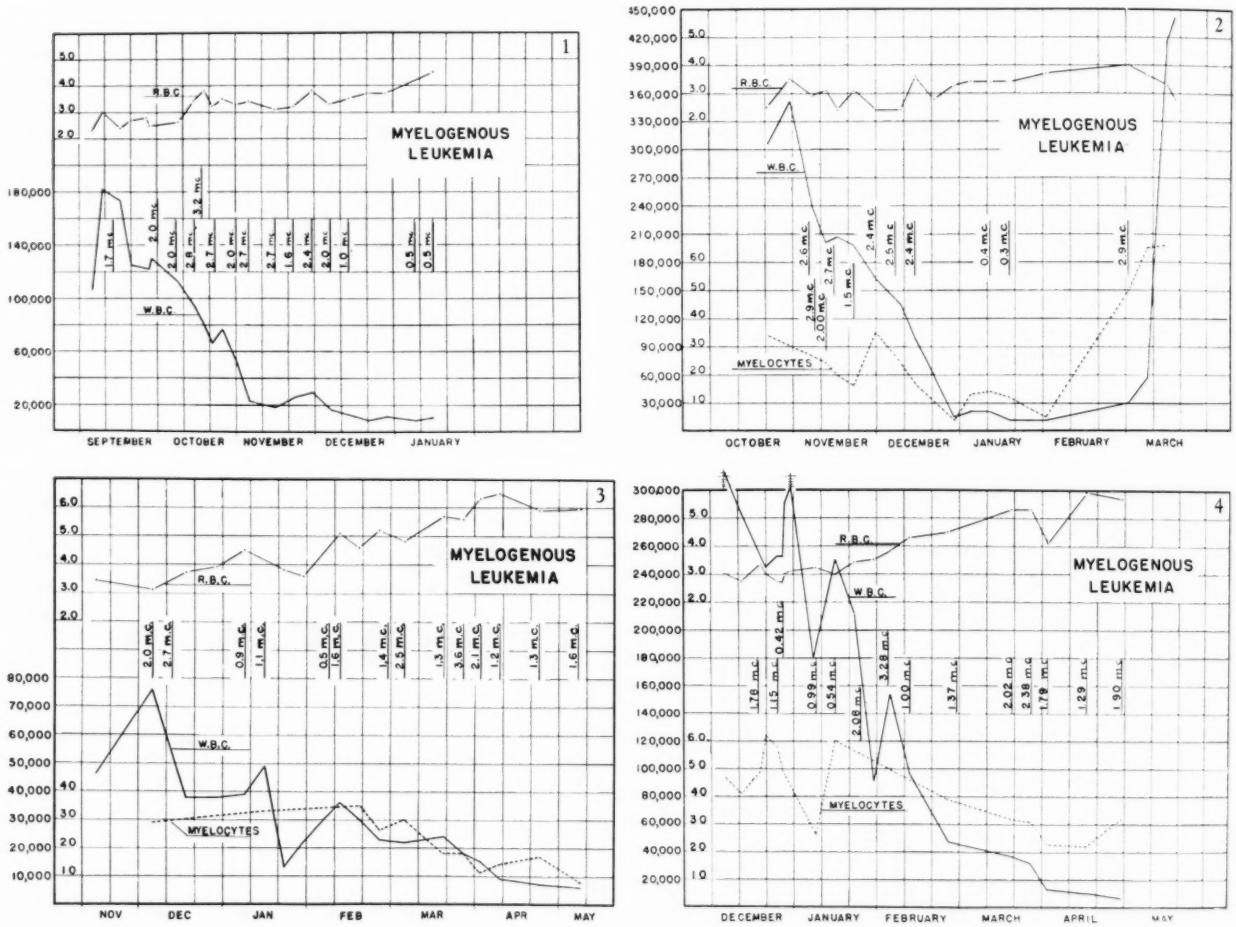


Fig. 1.—Regression of the leukocyte count and concurrent rise in red corpuscle count in a case of myelogenous leukemia treated with P^{32}. The red blood cell count in millions is given in the upper box to the right of the ordinate. The total leukocyte count is denoted by the figures to the left of the ordinate. Administered P^{32}, in mc., is shown in the center of the graph.

Fig. 2.—Regression of the leukocyte count in a case of myelogenous leukemia (Case No. 61153) treated with P^{32}. Resumption of therapy at the onset of an acutely progressive phase of the disease did not prevent a rapid increase in the leukocyte count and death. Red blood cell count in millions, upper right of ordinate; total leukocyte count, left of ordinate; per cent of myelocytes in the differential count, lower right of ordinate.

Figs. 3 and 4.—Regression of the leukocyte count and concurrent rise in the red corpuscle count in cases of myelogenous leukemia treated with P^{32}.

whose bone marrow show good erythropoietic tissue, receive an initial course of from 70 to 100 μc. per kg. of body weight. For patients with poor erythropoiesis, or those who are in the subacute phase of leukemia, the initial dose is reduced to 20 to 30 μc. per kg. of body weight, and may be even smaller for patients who are in the acute phase of leukemia. At the present time, it is not possible to decide upon a definite dose for each condition to be treated. Cases must be treated individually until more is known about the use of the isotope. The controlling factors should be the blood count, the marrow count, and the patient's general condition.

The present policy is to divide each course of therapy into 5 to 7 doses. This is based upon metabolic considerations. The phosphorus is, presumably, distributed as a normal metabolite, and its localization in any tissue will depend on that tissue's need for it. Apparently, leukemic and lymphosarcomatous tissue have a higher ratio of absorption than the average normal tissue. If, therefore, the total phosphate is given in small amounts repeatedly, rather than in one large amount, diseased tissues may absorb more of the radioactive phosphorus.

Limited experimental evidence appears to bear out this view. Two female patients with chronic myelogenous leukemia were treated by two technics. The first patient (Fig. 7) was given 10.2 mc. in a period of 10 days in 4 doses varying in size from 2.0 to 2.9 mc. The amount of phosphate administered at one time varied between 300 and 555 mgm. and totalled 1.76 gm. The peak activity of the leukocytes, 25 μc. per 100 cc., was reached 24 hours after the administration of the last dose of P^{32}. The second patient (Fig. 8) was given 8.1 mc. in a period of 10 days in 8 doses varying in size from 0.7 to 1.4 mc. The amount of phosphate administered each time was 60 mgm. and the total was 480 mgm. The peak activity of the leukocytes, 55 μc. per 100 cc., was reached 24 hours after the administration of the last dose of P^{32}. This peak was more than twice that seen in the first case. The time of administration in each case was the same, but the total amount of P^{32} administered to the second patient was less than that given to the first.

At the time of the peak value in the leukocytes for the first patient, the amount of retained P^{32} was 4.2 mc. In the second case, for the same point the retained dose was 4.9 mc. This difference in retained dose is not great enough in itself to account for the difference in the leukocyte values of radioactivity. It suggests that when small amounts of phosphate are administered repeatedly there is a higher absorption of the P^{32} by the leukemic tissue than when a single large dose is given. Whether this increase is due to the subdivision of the therapy or to the small amounts of total PO_4 administered, has not as yet been established. The retention of a greater percentage of the administered dose by the second patient than by the first also shows the importance of the administration of small amounts of phosphate in preventing undue loss due to excretion. This is discussed in detail below.

Careful measurements of the amounts of P^{32} excreted in the feces and urine have been made on a number of patients. These patients have received different amounts of phosphate at different intervals between doses. It has been consistently noted that, if the total phosphate administered in a single dose is less than 100 mgm., the total excretion during the next 96 hours is less than 20 per cent of the administered dose. This holds true when daily doses are given for 5 or 6 days. When the total phosphate administered in a single dose is 200 mgm. or more the excretion is usually above 30 per cent and may reach 50 per cent. The difference is in the amount excreted in the feces. Usually between 7 and 15 per cent of the administered dose, average 10 per cent, is excreted in the urine in the first 96 hours. This fraction increases somewhat as the amount of administered phosphate increases. The per cent excreted in the feces rises abruptly as the amount of phosphate is increased. With small doses it varies from 3 to 7 per cent; with amounts in excess of 150 mgm. of phosphate, it varies from 20 to 40 per cent. The daily fecal excretion of the P^{32} after the initial 96-hour period is very small, less than 0.3 per cent of the retained body dose. The urinary excretion after the initial 96-hour period varies from 0.4 to 0.6 per cent of the retained dose each day. Excretion measurements have also been made on patients who received the P^{32} by the intravenous route. The urinary excretion in these patients for the same amount of phosphate was comparable to that seen after oral administration. The fecal excretion was less than 1 per cent.

Succeeding courses of therapy are usually, though not necessarily, smaller than the initial course. Here the response of the individual patient is the deciding factor. Usually 7 to 10 days elapse between the completion of one course of therapy and the commencement of another. The optimum total amount to be administered is not as yet determined, but it would seem probable that it will be in the neighborhood of 500 μc. per kg. of weight. Here again more experience is needed before this point can be determined. The effect of the time factor, which is still poorly understood in x-ray therapy, is totally unknown in radioactive phosphorus therapy.

Myelogenous Leukemia

Eight patients with myelogenous leukemia, of whom 4 are living and 4 are dead, have been treated with

radioactive phosphorus. Six were in the chronic phase of the disease, one was in the subacute, and one in the acute phase.

One patient in the acute phase of the disease died one week after the administration of 1.5 mc. of P^{32}. He had a platelet count of about 30,000, widespread purpura, and 95 per cent myeloblasts in his bone marrow.

One patient in the subacute phase of the disease died 6 weeks after the commencement of therapy. Aspiration biopsy of the bone marrow before therapy showed poor erythropoietic tissue and beginning aplasia. The terminal picture was one of complete marrow aplasia. He received 3 doses of 0.8 mc., each at weekly intervals. The relationship of badly damaged marrow to failure of therapy is discussed later.

One patient with chronic myelogenous leukemia died 4 months after the start of therapy. There had been excellent hematological response to the isotope (Fig. 1) and the patient's general condition at his last clinic visit was good. He died suddenly at home and the story, as given by his family doctor, suggested a splenic and a pulmonary infarct. He had received 29 mc. (490 μc. per kg.) in 3 months' time.

The history and course of the fourth patient who died is as follows:

Case No. 61153. (Fig. 2.) A 24-year-old, white female, admitted on October 21, 1940. No contributory data were obtained from the past history or family history.

One year before admission, the patient discovered a mass in upper half of the left side of her abdomen which gradually increased in size. There were no other symptoms.

The findings on admission were as follows: The spleen extended 3 cm. to the right of the midline and to within 5 cm. of the symphysis pubis. The liver extended 3 cm. below the costal margin. Hemoglobin, 50 per cent; red corpuscles, 2,600,000; leukocytes, 307,200. Differential blood count: myelocytes, 33 per cent; polymorphonuclears, 44 per cent; mast cells, 8 per cent; eosinophiles, 4 per cent; monocytes, 9 per cent; lymphocytes, 2 per cent. Material from sternal marrow aspiration on October 30, showed: myelocytes, 55 per cent; myeloblasts, 20 per cent.

Course and treatment: From 11/6/40 to 11/15/40 the patient received four doses of P^{32} totalling 10.2 mc. From 11/22/40 to 12/14/40 she received, at weekly intervals, four doses that totalled 8.7 mc. There was excellent reduction of the leukocyte count by 12/14/40, and the spleen had regressed to within 1 cm. of the costal margin. On 2/1/41, the white count was 12,000 and the spleen just palpable. On 3/1/41, the white count had risen to 31,000 and the spleen had enlarged and filled the left upper abdominal quadrant; 2.9 mc. of P^{32} were administered. On 3/8/41, the white count was 56,000 and the spleen slightly larger. The patient was admitted to the hospital. X-ray therapy was instituted 3/10/41, with 250 kv., 70 cm. T.S.D.: 15 r (air) were administered daily over the spleen for 3 days. On 3/15/41, the white count was 414,000 and the spleen filled over two-thirds of the abdomen. She was then given 500 mgm. hours daily with the radium element pack at 15 cm. from 3/15 to 3/18. There was no improvement and the patient died 3/19/41. Autopsy was not permitted.

This case clearly illustrates that a patient may pass into the rapidly progressive phase of leukemia just at the time when apparently the therapy has been highly efficacious. It is to be noted that partial continuation of the therapy did not arrest the progress of the disease. The change to external radiation was made to increase the rate of local radiation therapy.

Four patients who were in the chronic phase of the disease are living, 16, 7, 5, and 4 months respectively, after the commencement of therapy. There has been marked improvement in the general condition of these patients. In each case the enlarged spleen has regressed, and in only one case is it, at present, more than barely palpable.

The patient surviving 7 months (Fig. 3) has received 27 mc. (365 μc. per kg.) in that period. The white count is now normal, but the per cent of myelocytes in the smear is still high. It is hoped by additional therapy to reduce further the number of myelocytes. His spleen is barely palpable. Of great interest is this patient's markedly increased erythrocyte count.

The patient who was first treated 5 months previously (Fig. 4) received 25 mc. (420 μc. per kg.) in that time. His white count became normal, but a number of myelocytes were observed in the blood smears. Further therapy is to be administered. His spleen is not palpable.

The patient who was first treated 4 months before the writing of this report (Fig. 5) has received 26 mc. (480 μc. per kg.) during that period. The reduction of the leukocyte count and the regression of the spleen have been slower than in the other patients. The spleen is about 2 cm. below the costal margin.

The history and course of the first patient to be treated is as follows:

Case No. 58868. (Fig. 6.) A 41-year-old, white male, admitted on January 27, 1940.

The patient's mother died of leukemia at 72.

The present illness began about 5 years ago with profuse bleeding after extraction of teeth, and a marked tendency to ecchymosis after trivial injuries. There had been vague abdominal discomfort for several months. Six weeks before admission he had a tooth extracted. This was followed by severe bleeding for 8 days, and admission to a hospital was necessary. A diagnosis of myelogenous leukemia was made at that time.

The findings on admission were: the spleen extended 4 cm. to right of the midline and to the crest of the ilium. The liver was not palpable. Hemoglobin was 65 per cent; red blood corpuscles, 3,400,000; white corpuscles, 310,000.

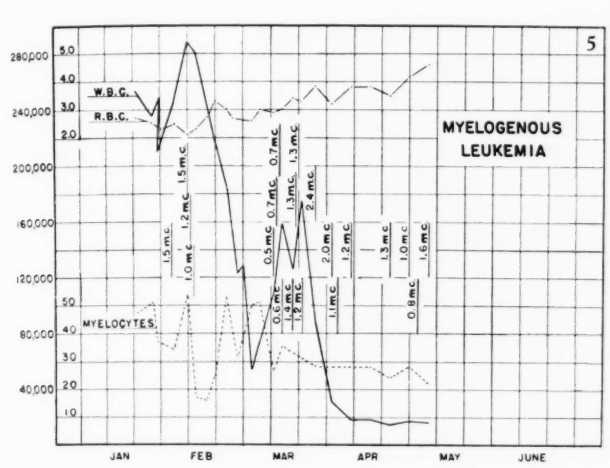
The differential count: myelocytes, 27 per cent; polymorphonuclear leukocytes, 47 per cent; mast cells, 3 per cent; monocytes, 20 per cent; lymphocytes, 2 per cent.

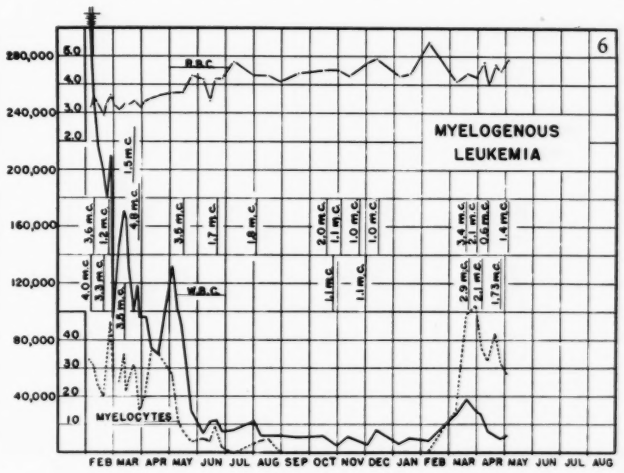
Course and treatment: From 2/26/40 to 3/27/40, at intervals of from 2 to 14 days, the patient was given 7 doses of P^{32} totalling 21.8 mc. Three additional doses at intervals of about 6 weeks were then given, raising the total dose to 28.8 mc. This reduced the white count to normal levels and decreased the size of the spleen to about 1 cm. below the costal margin.

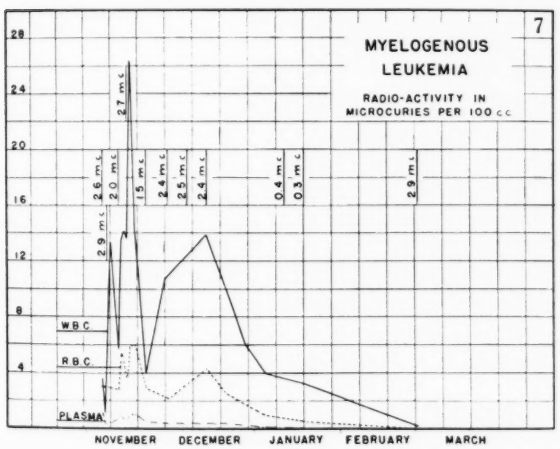
A marrow aspiration on 10/10/40 showed 60 per cent myelocytes. Between 10/19/40 and 12/14/40, at 7- to 14-day intervals, the patient was given 6 doses of P^{32} totalling 8 mc. This reduced the myelocytes in the marrow to 30 per cent. The white count remained normal and the spleen nonpalpable until 3/5/41. At that time the count rose to about 30,000 with 35 per cent myelocytes and the spleen was palpable 3 cm. below the costal margin. From 3/19/41 to 5/3/41, at 7- to 14-day intervals, he was given 7 doses of P^{32} totalling 14 mc. This reduced the size of the spleen and brought the white count down to normal levels. The myelocyte count in both the peripheral blood and the bone marrow, however, remained high. Further therapy is to be administered.

This patient had an excellent initial response to therapy. A recurrence has been well controlled by additional therapy.

Earlier in this paper several factors were given which permitted an estimation of the tissue dose of radiation in roentgen equivalents as administered by radioactive phosphorus. Figs. 7 and 8 show the radioactivity in the blood of two of the patients treated. At the level of 20 μc. per 100 cc. of leukocytes, assuming that 1 cc. of leukocytes weighs 1 gm., the white cells received between 8 and 9 "roentgens" daily. Analysis of Fig. 7 shows that in the period between November 9th and December 21st, the patient's leukocytes received about 180 "roentgens." Another comparison with x-ray therapy is possible on the basis of whole body radiation per mc. retained in the body (of a 70 kg. adult) for 24 hours. If decay and excretion are taken







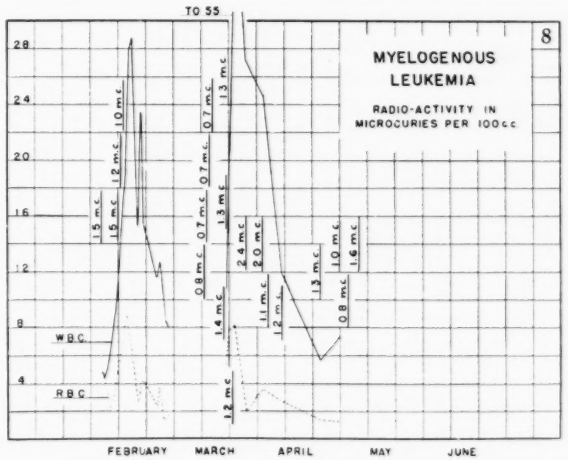


Fig. 5.—Regression of the leukocyte count with concurrent rise in the red corpuscle count in a case of myelogenous leukemia treated with P^{32}.

Fig. 6.—Regression of the leukocyte count and concurrent rise in the red corpuscle count in a case of myelogenous leukemia (Case No. 58868) treated with P^{32}. Note the absence of myelocytes during a period of 6 months, September to February, and that an exacerbation of the disease responded to additional therapy.

Fig. 7.—The radioactivity of the leukocytes, red corpuscles, and plasma secured by centrifuging oxalated blood, in a case of myelogenous leukemia treated with P^{32}, is shown in μc. per 100 cc. of each blood fraction. Values from the middle of November on are for fractions of blood drawn just before administration of P^{32} and one week after the last dose of P^{32}. Note the marked differences between the radioactivity of leukocytes and red corpuscles.

Fig. 8.—Radioactivity in μc. per 100 cc. of leukocytes, red corpuscles, and plasma from blood of a patient with myelogenous leukemia treated with P^{32}. Values after April 1 are from blood taken just before administration of P^{32}, and one week after the last dose of P^{32}. The marked differences between the radioactivity of leukocytes and red corpuscles are clearly shown.

into account, it is possible to derive an approximate whole body radiation dose in terms of "roentgens." For the patient of Fig. 7 this would amount to 240 "roentgens" for the period between November 6th and January 18th. It is to be remembered, however, that the radioactive phosphorus is not distributed evenly throughout the body. Therefore, the estimation of whole body therapy due to radiation from radioactive phosphorus is not exactly comparable to that from whole body x-irradiation. If x-radiation is used, all tissues at the same depth receive equal doses. When radioactive phosphorus is the source of whole body radiation, the tissues are irradiated unevenly, and for leukemia, the involved tissues receive a larger dose than do normal tissues.

Table IV shows the unequal distribution of phosphorus in leukemia. It contains the measurements made on tissues from patients who died after therapy and were autopsied. The difference between identical tissues with different degrees of leukemic infiltration is, in some instances, striking and is best illustrated by comparing the values for kidney and testis of cases No. 59290 and No. 60554, or by comparing values in case No. 61134 for two pieces of kidney, one of which had marked, and the other no, leukemic infiltration. The amount of radiation, in roentgen equivalents, administered to these tissues can be estimated from these measurements. The estimations probably would not be accurate to more than plus or minus 15 per cent, because the values for the days preceding death can only be calculated by allowing for the decay in radioactivity. Loss in these tissues due to excretion or gain due to absorption, during this period, is unknown. The figures do suggest, however, that absorption is rapid and early, and that the absorbed amount is largely retained for at least 8 days. This is also borne out by tissue measurements in patients with diseases other than leukemia, in whom biopsy or surgical specimens were removed at varying periods after administration of the radioactive phosphorus. If this is true, it is of greatest importance, because the largest and most significant part of the radiation from this isotope is administered during the first 3 weeks after any given dose (10).

TABLE IV: LEUKEMIA: RADIOACTIVITY OF TISSUES IN PATIENTS AUTOPSIED

Case number	59290	61134	60554	61435	62061
Age and sex	3 yrs., M.	13 yrs., F.	61 yrs., M.	6 yrs., F.	19 yrs., M.
Diagnosis	Lymphatic leukemia	Lymphatic leukemia	Lymphatic leukemia	Lymphatic leukemia	Myelogenous leukemia
Amount of P^{32} administered in mc., and date	8/2 0.4 8/7 0.6 8/14 0.2 8/20 0.6 8/22 0.7 8/30 0.8 9/4 0.8 9/8 0.5 9/13 0.6	10/31 1.0 11/5 1.0 11/9 1.0 11/12 1.2	8/20 2.8 8/24 3.4 8/28 3.2	11/30 1.1 12/1-12/6 incl. 0.63 mc. daily 12/16 0.7	3/3 1.4
Total P^{32} administered in mc.	3.6	5.4	9.4	5.6	1.4
P^{32} in body at death (estimated), in mc.	1.2	2.3	4.6	1.6	1.1
Date of death	9/16/40	11/19/41	9/4/40	12/24/40	3/4/41

Tissue	μc. per kg.	L.I.*	μc. per kg.	L.I.*	μc. per kg.	L.I.*	μc. per kg.	L.I.*	μc. per kg.	L.I.*
Liver	120	3	137	3	138	3	112	0	112	3
Spleen	107	3	145	3	112	3	98	1	69	3
Lymph Node	131	3	117	3	82	3	101	1	61	3
Marrow	..	...	147	3	...	...	112	1	...	...
Vertebrae	102.1	...	113	...	93	...	285(rib)	...	53	...
Kidney	112.0	3	68 143	0 3	21	1	85	...	80	2
Muscle	45.1	...	27	...	34	...	60	...	10	...
Heart	65	0	50	...	...	...	99	...	...	2
Lung	64	2	56	2	46	...	47	0	...	...
Pancreas	85	1	50	1	...	...	...	...	35	...
Intestine	89	...	54	...	49	...	64	...	...	...
Stomach	64	1	39	1	26	...	76	0	...	...
Testis	132	3	...	...	57	1	...	...	...	...
Ovary	..	...	80	...	...	...	...	...	...	...

* L.I. = Leukemic infiltration with grade denoted by numeral.

A careful study of the patients with chronic myelogenous leukemia has led to the conclusion that radioactive phosphorus is an effective therapeutic agent in this disease because: 1. It reduces the white count to normal, or nearly normal (6 of 6 patients). 2. Enlarged spleens regress (6 of 6 patients). 3. Erythropoiesis is not disturbed, and the hemoglobin and erythrocyte count rise during therapy (6 of 6 patients). 4. It reduces the percentage of myeloblasts and myelocytes in the marrow (see section on Bone Marrow). 5. There is no radiation sickness during P^{32} therapy (6 of 6 patients).

The failure of P^{32} to influence the course of the disease in the patients in the subacute and acute phases may mean that the isotope would be of no more value in these phases than any other form of radiation.

Lymphatic Leukemia of Childhood

This is usually an acute type of leukemia which is notorious for its early and fatal outcome. Radiation therapy of any type has been uniformly unsuccessful in the treatment of these patients. Eight children with acute or subacute lymphatic leukemia have been treated with radioactive phosphorus. Seven are dead, all except one within 4 weeks from the time treatment began. The one patient still living was only partially treated before he was removed from the hospital. The therapeutic effect of the isotope in this patient is open to question.

Careful analysis of the results of the treatment of this group of patients has led to the following conclusions: Radioactive phosphorus therapy has to date been no more effective in the acute or subacute leukemia of childhood than has any other form of radiation therapy; *i. e.,* had no value at all.

Lymphatic Leukemia in Adults

Eight patients have been treated with radioactive phosphorus. Four are dead, 8, 4, 4, and 2 weeks after the commencement of therapy. One died following a splenic and pulmonary infarct. He was in the aleukemic phase with a leukocyte count of from 2 to 3,000 and had been treated for 2 months with some improvement, as judged by the size of the spleen, red count, and general condition. One patient had had extensive x-ray therapy for 2 years and was in the terminal phase of his disease when radioactive prosphorus therapy was attempted. The third and fourth patients had long histories and had had a large amount of x-ray therapy previously. In the last 3 patients no change was noted in the size of the spleen or lymph nodes. At the time isotope therapy was started, their sternal marrow biopsies showed badly damaged tissue. This relationship of badly damaged marrow to therapy failure is discussed later.

Four patients are living, 6, 6, 6, and 4 months, respectively, after the commencement of therapy. The patient whose first treatment was administered 4 months before this report was written (Fig. 9) had just finished a full course of x-ray therapy, but without complete control of his disease. He has received 16.1 mc. of P^{32} in 120 days with a reduction of the leukocyte count from 20,000 to 7,000. There are no palpably enlarged lymph nodes and the spleen is only barely palpable. There has been some recent improvement in the differential count. Two of the 3 patients who have been under treatment for the past 6 months have long histories and have had a moderate amount of x-ray therapy. The disease in one patient was well controlled, in the other patient only partially controlled, and it was necessary to administer x-ray therapy to a group of enlarged lymph nodes. The history of the fourth patient follows:

Case No. 61314. (Fig. 10.) A 58-year-old, white male, admitted 11/15/41. There were no contributory factors found in the family history. The patient was a chronic alcoholic with a mild addiction to barbital. Progressive weakness and fatigability had existed for 3 years. Enlarged lymph nodes in neck and axillae had been present for 3 years. The patient had frequent attacks of lower abdominal pain radiating to both flanks during 18 months. In June, 1940, chronic lymphatic leukemia was diagnosed, but no therapy was prescribed.

The findings on admission were: Generalized enlargement of lymph nodes in the neck, axillae, and groin, none of which exceeded 1.5 cm. in size. The liver was palpable 1 cm. below the costal margin and the spleen palpable 2 cm. below costal margin. Hemoglobin, 82 per cent; red corpuscles, 4,100,000; white corpuscles, 27,200.

The differential count showed: polymorphonuclear leukocytes, 15 per cent; eosinophiles, 2 per cent; monocytes, 3 per cent; lymphocytes, 80 per cent.

Marrow aspiration on 11/15/40 showed: lymphoblasts, 18 per cent; lymphocytes, 40 per cent; erythroblasts, 6 per cent; normoblasts, 10 per cent; myelocytes, 11 per cent; polymorphonuclears, 6 per cent; metamyelocytes, 5 per cent; eosinophiles, 4 per cent.

Course and treatment: The patient received 7 doses, totalling 9.1 mc., at 7 to 14 day intervals between 11/19/40 and 1/20/41. During this time there was no significant change in the white count. There was some regression in the size of the lymph nodes and spleen. On 1/27/41, the patient complained of acute left upper quadrant pain of 4 days' duration. A diagnosis of splenic infarct was made. Recovery was complete after 7 days' bed rest. From 3/4/41 to 4/16/41 the patient received, at weekly intervals, 6 doses totalling 11.2 mc. There was still no significant change in the white count. On 4/16/41, there were no enlarged lymph nodes and the spleen was only slightly palpable. The patient's general condition was excellent.

Radioactive phosphorus therapy has not altered this patient's white count. It has caused a regression in size of the enlarged lymph nodes and spleen. Symptomatically, the patient has improved markedly.

Analysis of the group of 8 patients with chronic lymphatic leukemia who have been treated with P^{32} has led to the following conclusions: 1. It should be

administered cautiously to patients with aplastic or infiltrated bone marrow. 2. It has not been effective in cases that have become radiation-fast. 3. It reduced the enlarged lymph nodes in 4 patients and reduced the enlarged spleens in 3 patients. 4. The leukocyte count was decreased in 1 of 8 patients. 5. The proportion of lymphocytes in the differential count was altered in 1 of 8 patients. 6. Symptomatically, 5 of the 8 patients were improved. 7. There is no radiation sickness.

Lymphosarcoma

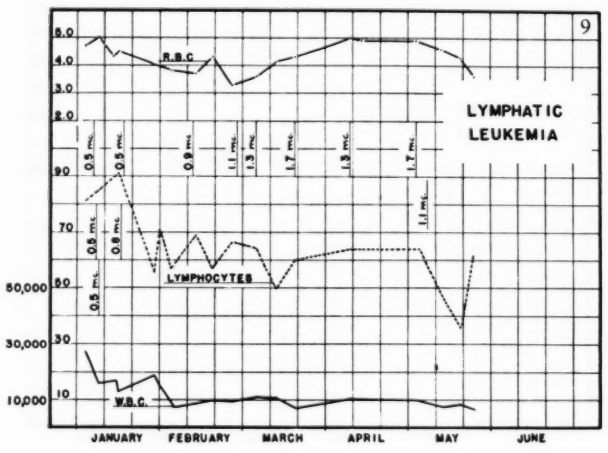
A group of 12 patients with a biopsy diagnosis of lymphosarcoma have been treated with radioactive phosphorus (5), and of these, 7 have been observed for a sufficient period of time to permit some evaluation of the benefits of the therapy in their cases. There has been good regression of the enlarged nodes in all of these cases except one. In 5 of the cases, this regression has been complete. In 1 patient there has been complete regression of enlarged neck nodes and about 50 per cent regression of enlarged axillary nodes. The patient whose disease did not regress actually showed enlargement of his nodes while therapy was being administered.

During therapy, some of the patients have shown moderate depression of the blood count, particularly the leukocyte count. In only one has there been a serious depression, and in this instance the marrow showed marked lymphocytic infiltration before therapy was started. Shortly after therapy was complete, the blood count began to fall and marrow biopsy showed aplastic tissue. Repeated transfusions have kept the patient in good condition, and a recent marrow aspiration has shown beginning regeneration.

The method of therapy in these patients has varied, but recently a more stable procedure has been outlined, to provide between 60 and 90 μc. per kg. as an initial course of therapy. This dose is divided into 5 to 7 portions administered on successive days. After a week or ten days, the course is repeated, though usually the second total dose is somewhat smaller. Successive courses follow at 10-day to 2-week intervals until about 300 μc. per kg. of weight have been administered. The plan of therapy depends on the patient's blood count, bone marrow, and responses to treatment. Each patient must be handled as an individual problem.

While all these patients have been treated only with radioactive phosphorus, it is believed that the greatest use for this isotope will be as an adjunct to x-ray therapy. The isotope has been used alone in these cases in order that some estimation might be made of its effect on lymphosarcoma. It is probable that the best method would be to treat the enlarged node masses with x-rays in the usual manner and then also to administer radioactive phosphorus either during or after the x-ray therapy. This method would destroy rapidly the obviously diseased tissue and might lead to destruction or inactivation of the small foci of disease in nonenlarged nodes. If therapy with radioactive phosphorus is combined with x-irradiation, it should be administered slowly and the blood count should be watched carefully.

Only 7 months have elapsed since the first patient was treated. Hence, no estimation of the end results is possible at this time. A careful study of these cases does permit, however, the following conclusions: 1. Lymphosarcoma nodes apparently will completely regress under radioactive phosphorus therapy (6 of 7



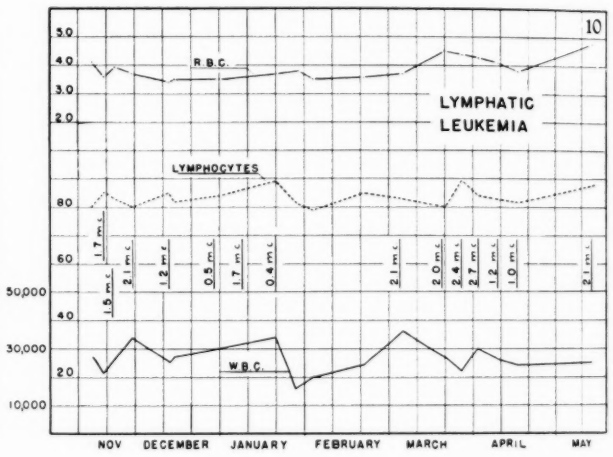


Fig. 9.—Regression of leukocyte count and partial regression of the per cent of lymphocytes in a case of lymphatic leukemia (Case No. 61314) treated with P^{32}.

Fig. 10.—A case of lymphatic leukemia treated with P^{32} with virtually no change in either the leukocyte count or the per cent of lymphocytes.

patients). 2. There is some depression of the blood count during therapy, but, when the marrow has been normal at the outset, the damage has not been serious. 3. Radioactive phosphorus should prove to be a valuable adjunct to x-ray therapy in the treatment of lymphosarcoma.

Osteogenic Sarcoma

Eight patients with osteogenic sarcoma are receiving radioactive phosphorus therapy. In all but one patient this has been administered prophylactically with the hope that it will be absorbed sufficiently by the probably present, though clinically nonevident, metastases to damage them enough to prevent their growth. x-ray therapy. As was mentioned earlier, deposition of radioactive phosphorus in these tumors is largely a function of their alkaline phosphatase content (12). This phosphatase activity is largely inactivated by the preoperative x-radiation (13). It is not at all unlikely, therefore, that the tumor accumulation and tumor roentgen equivalent dose shown in this table do not represent what the irradiation may be in the distant metastases. In this respect, it is to be remembered, however, that distant metastases do not always have the same phosphatase activity as the primary tumor. Another year must elapse, and more patients must be treated, before any evaluation of radioactive phosphorus therapy in osteogenic sarcoma can be made.

Table V: Osteogenic Sarcoma; Tissue Absorption After Therapeutic Amounts of Radioactive Phosphorus

Case number	Amount of P^{32} and time	Interval from first dose P^{32} to operation	Estimated P^{32} in body at operation (excretion & decay subtracted from administered dose)	μc. per kg. body weight		Differential absorption ratio and estimated roentgen dosage from P^{32}						X-ray therapy tumor dose in "r" units
						Tumor		Normal bone		Muscle		
				Administered	Remaining at operation (estimated)	Ratio	Equivalent roentgen	Ratio	Equivalent roentgen	Ratio	Equivalent roentgen	
61863	5 doses in 34 days totalling 4.5 mc.	61 days	9.6 mc.	218	29	3.6	600	...	...	0.5	85	4,700 in 20 days
60931	4 doses in 29 days totalling 10.96 mc.	34 days	2.5 mc.	233	53	3.8	400	2.0	200	0.7	75	3,700 in 32 days
61944	7 doses in 24 days totalling 10.71 mc.	28 days	3.3 mc.	195	60	4.3	400	0.9	85	0.7	65	6,450 in 26 days
61059	12 doses in 115 days totalling 20.8 mc.	123 days	2.1 mc.	320	33	2.4	400	0.7	120	1.1	185	4,000 in 22 days

All but one of these patients are still being treated, and no estimation of end results of this therapy is possible. One patient who had refused amputation and whose primary tumor had received a 9,100 "r" tumor dose of 1,000 kv. x-ray at 70 cm. T.S.D., was given 20 mc. over a period of 3 months. Treatment was discontinued because of the growth of extensive metastases during the period of phosphorus administration.

Four of the remaining 7 patients were operated upon after they had received a portion of their isotope therapy. Table V presents a résumé of their therapy and the measurements of radioactivity in the surgical specimens. It is to be noted that all of these patients had received large doses of x-ray therapy before operation. Included in the table is an estimation of the roentgen equivalents delivered to the tumor by the phosphorus. This "roentgen" dose is not encouraging. There may be a partial explanation for these low values in that the tumor had been largely inactivated by the

Miscellaneous Cases

This group is made up of 5 patients, each with a different type of malignant neoplasm, as follows: metastasizing hemangioma; lymphoepithelioma of the tonsil with widespread metastasis; melanoma with numerous cutaneous metastases; extensive mycosis fungoides; widespread multiple myeloma.

The patient with the metastasizing hemangioma received a total dose of 150 μc. per kg. of body weight in 18 days. This was divided into small daily doses in order to maintain the blood concentration of the isotope at as high a level as possible. It was hoped that by so doing sufficient irradiation would be administered to the walls of the blood vessels which comprise the lesions to cause some regression. No regression was observed during life, nor were any changes noted in the tumors at autopsy, 20 days after the commencement of therapy, that could be attributed to the P^{32}.

Three hundred and fifty μc. per kg. of body weight were administered in divided doses in 34 days to the patient with metastasis from a lymphoepithelioma of the tonsil. No regression of these lesions was noted at any time.

The patient with cutaneous metastasis from a melanoma received 350 μc. per kg. of body weight in divided doses in 40 days. There was a steady growth of all of the lesions during and after the administration of the isotope. Measurements of radioactivity of a lesion removed at the close of therapy showed good concentration of the P^{32}.

The patient with mycosis fungoides received 200 μc. per kg. of body weight in 19 days. There was some regression of the disease. Unfortunately he left the hospital before therapy was considered complete and his course since that time is not known. Because some regression of disease was noted in this case, it might be advisable to consider P^{32} therapy when it is proposed to treat a patient with mycosis fungoides.

The patient with multiple myeloma received 250 μc. per kg. of weight in 4 months' time. This therapy was divided into several courses and administered at irregular intervals. It was observed that during the first 3 months there was marked diminution in the amount of pain, and improvement in the patient's general condition. There was no regression of the lesions as determined by x-ray pictures. The patient died in an exacerbation of his disease 7 months after the institution of the therapy. The results noted in this case indicate that P^{32} may be a useful palliative method of therapy in patients with multiple myeloma, particularly if the disease is widespread.

Bone Marrow

Careful study of the bone marrow, as secured by aspiration, has been made in most of the cases treated by radioactive phosphorus. This has been of importance because in the cases of leukemia it has provided some index of the success of the therapy, and in the cases of lymphosarcoma and osteogenic sarcoma it has given information as to how the blood-forming organs tolerate therapy.

All of the patients with chronic myelogenous leukemia have shown a decrease in the percentage of myelocytic and myeloblastic elements in the marrow. In most of these cases, there has been a corresponding increase in the erythropoietic tissue and in the more mature forms in the myelocytic series. Patients with a high initial percentage of myeloblastic elements and with a low erythropoietic percentage have not tolerated treatment with the radioactive phosphorus.

In chronic lymphatic leukemia, there has been very little change seen in the bone marrow during the therapy. There has been some decrease in the lymphocytic and lymphoblastic elements, but this has not been marked.

The marrow taken from patients with lymphosarcoma or osteogenic sarcoma has shown some suppression of the myelocytic elements during the course of therapy. In general, this has not been serious.

It has been consistently noted that patients with damaged bone marrow, in the sense that there is heavy infiltration with abnormal cells, or beginning aplasia, do not tolerate therapy with radioactive phosphorus. Patients with this type of marrow tissue should be treated cautiously or not at all.

Measurements of the radioactivity of the bone marrow, as secured by sternal aspiration, have been made on many of the patients receiving P^{32}. Table VI presents the essential data on this group of patients, and also contrasts the radioactivity of the marrow with that of whole blood. It is appreciated that marrow secured by aspiration is diluted to some extent by blood and that, therefore, the values for marrow in the table are somewhat lower than their true value. This dilution was reduced to the minimum by limiting the aspiration to 0.5 to 0.7 cc. of marrow. In myelogenous leukemia the activity of the marrow is 3 to 5 times that of whole blood. This is significant because it means that one of the primary sites of infiltration in myelogenous leukemia absorbs adequate amounts of the isotope.

In only a few of the patients with diseases other than leukemia was the activity of the marrow as much as twice that of whole blood. In many of these patients, marrow and whole blood radioactivity were about equal, and in several of the patients, the activity of the blood was greater than that of marrow. Cases No. M8049 and No. N9268 are of especial interest because they had received large amounts of P^{32} only a few days before their marrow biopsy. In these two patients there was less radioactivity in the marrow than in the blood.

Cytological Studies

Histological examinations were made of tissues from patients who had received radioactive phosphorus for various diseases. The material studied consisted of lymph node tissue removed at biopsy and tissues obtained at necropsy. In several cases, lymph nodes excised before treatment was begun were available for comparison with excised nodes that had shown clinical regression under therapy.

No cytological changes in the nodes were found that could be definitely attributed to the action of the radioactive isotope. In one node, partial hyalinization of the germinal centers was present, together with some fibrin deposit, but as these changes were ob-

served in nodes from other untreated cases, their origin is obscure.

Careful examination of organ tissues and bone marrow secured at autopsy from cases of leukemia failed to reveal cellular necrosis or other changes ascribable to the action of P^{32}, except in one case. In this case, of acute lymphatic leukemia, the terminal bone marrow picture was one of extreme aplasia with multiple hemorrhages. The marrow changes were more severe than those ever seen in this institution in terminal leukemia not treated with P^{32}. Furthermore, they simulated closely the changes produced in the marrow of normal mice by massive doses of P^{32}.

While the above changes are suggestive, we have not seen enough well-controlled material from patients receiving appreciable quantities of P^{32} to come to any definite conclusions as to the histological alterations produced by this substance.

TABLE VI: RADIOACTIVITY OF BONE MARROW

Case number	Diagnosis	Degree of marrow infiltration	P^{32} dose and time		Last dose of P^{32} and date		Radioactivity		
			Mc.	Days	Mc.	Days previous	Marrow μc./kg.	Whole Blood μc./kg.	Ratio marrow to whole blood
60993	Myelogenous leukemia	Moderate	4.7	7	2.7	5	48.4	15.0	3.2
		Moderate	4.7	7	2.7	24	19.0	4.0	4.8
61491	Myelogenous leukemia	Marked	4.3	21	1.0	7	30.4	8.0	3.8
		Moderate	19.8	140	1.8	14	20.6	6.2	3.3
N4354	Lymphosarcoma	Slight	10.6	99	1.2	24	11.5	5.4	2.1
L5544	Lymphosarcoma	Slight	11.7	72	2.9	7	22.8	11.7	1.9
		Slight	19.4	100	1.6	7	17.2	14.6	1.2
M8049	Lymphosarcoma	Slight	9.7	19	1.0	7	14.4	17.4	0.8
N9268	Lymphosarcoma	None	4.3	4	1.1	1	22.0	34.0	0.6
61647	Osteogenic sarcoma	None	4.6	21	0.4	1	18.4	10.8	1.7
59700	Osteogenic sarcoma	None	1.2	3	1.2	3	4.5	6.9	0.7
		None	6.9	77	1.0	7	7.3	5.6	1.3
		None	13.5	119	1.3	7	11.3	11.8	1.0

ALTERATIONS IN THE PHOSPHORUS METABOLISM IN LEUKEMIA AFTER P^{32}

During the course of an investigation of phosphorus metabolism in leukemia (1), it was noted that the administration of a small amount of P^{32} altered the values of chemically determined organic acid-soluble phosphorus in the leukocytes and erythrocytes by 50 to 100 per cent. Four patients with leukemia were put on controlled diets and after a period of time, 450 mgm. of *nonradioactive* phosphorus were administered. No significant alterations of the organic acid-soluble phosphorus levels in the blood cells were observed.

Each of these patients was then given about 1.5 mc. of P^{32} in the same amount of phosphate as was administered in the nonradioactive dose. In each instance, this produced a marked increase or decrease in the organic acid-soluble phosphorus[4] level in the leukocytes and usually in the erythrocytes. There was usually a marked agitation of the values over a period of 3 to 10 days. Fig. 11 shows the effect of the P^{32} on these compounds in one of the patients studied. All measurements are in mgm. of phosphorus per 100 cc. of cells. The method of Fiske and Subbarrow (3) was used in these determinations.

The belief that these alterations of phosphorus metabolism were due to the radiation delivered to the leukocytes and erythrocytes by the P^{32} was confirmed when the same changes were observed after the administration of x-rays, either to the whole body or directly to the circulating blood through a port over the heart. The amount of radiation that produced these changes was, in each instance, 3 "r" or less.

These observations are of great importance to all investigators who are using radioactive isotopes as tracers in metabolism studies. It is quite likely that some of the results of these studies are measurements of disturbances of metabolism due to irradiation and are not measurements of normal processes. This is particularly true of all such investigations with P^{32}. In view of this finding after the administration of P^{32}, all tracer work on such problems should be care-

[4] These values were determined by the usual technic of measuring blood phosphorus. They are all chemical measurements and were not determined by measuring radioactivity.

fully evaluated to be certain that the results represent physiological and not radiation metabolism.

The amount of isotope administered to these patients is the equivalent, on a weight basis, of about 1 μc. in a 30 gm. mouse. From 1 to 5 μc. is the usual tracer dose, for mice, in experimental investigations. Therefore, the amount of P^{32}, 1.5 mc., administered to these patients should be strictly comparable to the amount of isotope that is usually used in tracer work in mice.

SUMMARY AND CONCLUSIONS

A review has been presented of an 18 months' investigation by a group of workers at The Memorial

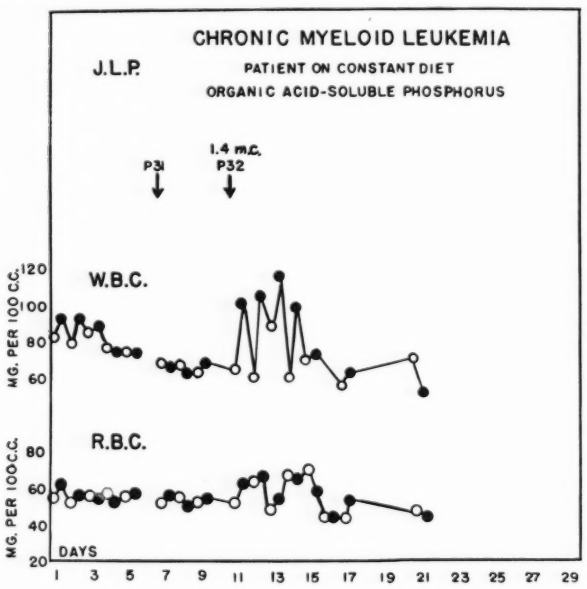


FIG. 11.—Changes produced in the organic acid-soluble phosphorus fraction of leukocytes and red blood corpuscles after the administration of 1.5 mc. of P^{32} to a patient with myeloid leukemia. Note absence of changes after administration of P^{31}.

Hospital on radioactive phosphorus as a therapeutic agent in malignant neoplastic disease. Its physical characteristics and the estimation of the radiation it delivers in tissue have been discussed.

A differential absorption ratio has been devised by means of which it is possible to estimate the relative absorption of the P^{32} by various tissues after administration of the isotope. It is based on the amount of the isotope administered per kilogram of weight as related to the amount absorbed by a kilogram of a given tissue.

Subtherapeutic amounts ("tracer doses") of radioactive phosphorus have been administered to patients with carcinoma of the breast, osteogenic sarcoma, and lymphosarcoma. Measurements of radioactivity were made on portions of the various tissues removed from these patients at operation. The ratio described above was then calculated in each instance. From the results obtained in this manner, it was learned that radioactive phosphorus was preferentially absorbed by the tissue of osteogenic sarcoma and of lymphosarcoma, and less so by carcinoma of the breast.

The method of the treatment of leukemia with radioactive phosphorus has been discussed. Six patients with chronic myelogenous leukemia (of whom 4 are still living) have been treated with P^{32}, and the isotope found to be a good and reliable therapeutic agent in this disease, because, in each case, a) it reduced the leukocyte count to nearly normal, b) the enlarged spleen was markedly reduced in size, c) the percentage of myelocytes and myeloblasts in the bone marrow was decreased, d) erythropoiesis was not seriously disturbed, and e) there was no radiation sickness.

P^{32} therapy has been used on 8 patients with chronic lymphatic leukemia, 4 of whom are still living. Five of the 8 patients improved symptomatically during treatment. Three patients had enlarged spleens, and 4 patients had enlarged lymph nodes. In each instance, these were reduced in size to normal, or nearly normal. The leukocyte count and differential count were not appreciably altered except in one instance. It would appear that P^{32} will be a useful therapeutic agent in this disease.

Ten patients in the acute, or subacute, phase of leukemia (2 myelogenous and 8 lymphatic) have been treated with P^{32}. All but one of these patients are dead, and in no case was there any improvement which could be attributed to the isotope. It is believed that radioactive phosphorus is not a useful method of therapy in this type of disorder.

Twelve patients with lymphosarcoma have been treated with radioactive phosphorus. Seven patients have been observed for a sufficient period of time to permit a preliminary evaluation of the results of the therapy. Five patients have had complete regression of the disease, plus freedom from recurrence for periods of 1 to 8 months. One patient has had partial regression of his disease. One patient did not respond to P^{32} therapy. There has been some depression of the blood count during therapy in all patients, but this was serious in only one instance. It is believed that the isotope will be a useful therapeutic agent in this disease, because it brings about regression of lymphosarcoma tissue and because it is distributed to all the areas in which such tissue is present.

Eight patients with osteogenic sarcoma have been treated prophylactically with P^{32}, with the hope that the probably present, though clinically nonevident, metastases would absorb a sufficient amount of the isotope to be inactivated by its radiation. Seven patients are still living, but sufficient time has not elapsed to justify even a preliminary evaluation of

results. One patient is dead. In this case, extensive metastases appeared during the period of administration of the isotope.

The cytologic studies made on tissue removed from patients treated with radioactive isotopes are briefly presented. No alterations of cellular characteristics were noted that could be definitely ascribed to P^{32}.

Careful studies have been made on the bone marrow of most of the patients treated with radioactive phosphorus. Marrows infiltrated with neoplastic tissue tend to absorb more of the isotope than noninfiltrated ones.

A change in the phosphorus metabolism of blood cells in patients with leukemia after the administration of subtherapeutic amounts of P^{32} has been described.

As stated at the beginning of this paper, the material presented is a summary of cooperative research.

Dr. Jules C. Abels developed and supervised the investigation of the alterations of the organic acid-soluble phosphorus compounds in leukocytes and red corpuscles in leukemia after radiation.

Dr. Lloyd F. Craver, of the Department of Medicine, contributed much to the success of the clinical application of P^{32}. His experience in radiation therapy of leukemia and lymphosarcoma has been a valuable asset.

Mr. L. D. Marinelli, of the Department of Physics, was in charge of all measurements of radioactivity. He has developed the methods used by us for estimating tissue doses of radiations from absorbed P^{32}.

Dr. Levin Waters, of the Department of Pathology, under the supervision of Dr. Fred Stewart, examined the tissues secured at operation or autopsy from patients receiving therapeutic amounts of radiactive phosphorus.

Dr. Helen Q. Woodard, of the Department of Chemistry, supervised or performed all the phosphatase measurements on blood and in tissue secured at operation or autopsy from patients with bone tumors who had received P^{32}.

The author is deeply indebted to Dr. Edith Quimby and Dr. G. Failla, of the Department of Physics, and to Dr. C. P. Rhoads, Director of the Memorial Hospital, for their many valuable suggestions during the preparation of the paper.

The careful, conscientious technical assistance of Miss Mildred Moore, Miss Douglas Taylor, and Miss Audrey Anschuetz have been invaluable in this work.

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Glycogen in Walker Tumor 256*†

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Subsequent to the observation of Schott, Samuels, and Ball (21) that the slow-growing Walker 256 tumors of hypophysectomized rats contained considerably greater concentrations of glycogen than were found in the more actively growing tumors of intact animals, it became of interest to determine whether a high glycogen percentage was associated generally with slow tumor growth or was a special effect of hypophysectomy.

The significance of glycogen in normal cells, whether static or proliferating, has never been satisfactorily defined. Its significance in neoplastic cells is, therefore, bound to be in doubt. Two conflicting views are held; one, that glycogen is most abundant in rapidly proliferating neoplasms, and the contrary, that it is more abundant in the relatively static growths.

Brault (5), using a histological technic, studied malignant tissues and found glycogen most abundant in the areas of greatest proliferation. He concluded that the degree of malignancy was proportional to the amount of glycogen present and reiterated his belief in 1938 (6). Gierke (10) made observations on a variety of tumors and concluded that glycogen was present on the same basis as its occurrence in normal cells. Lubarsch (14) observed more glycogen in sarcomas than in carcinomas, while Best (3) found glycogen to be more abundant in slow-growing tumors. Although further early observations are on record, little knowledge was added until after a chemical technic for determination was available (18) and interest in the subject was renewed.

Cori and Cori (7), using a modification of Pflügers chemical method, reported glycogen values for 6 spontaneous carcinomas in mice within the range of 0.171 to 0.303 per cent, and for 1 Jensen rat sarcoma as 0.122 per cent. They stated that ingestion of glucose seemed to increase the glycogen content of tumors. Tesauro (22) reported values for 5 cases of cancer of the cervix and 1 case of carcinoma of the fundus. These values averaged 0.759 per cent of the dry weight of the sample, corresponding to about 0.114 per cent of the fresh weight. Fahrig (8) also worked with human tumors. Bernhard (2) reported low values (0.014 to 0.066 per cent) for mammary carcinoma and myoma of the uterus, and high values (0.436 to 0.682 per cent) for carcinoma of skin, bladder, and stomach. The value for one spindle cell sarcoma is given as 0.172 per cent. Twelve tumors were analyzed and the conclusion was drawn that malignant tumors contain more glycogen than benign ones. Borghi (4) found an average value of 0.01 per cent for 5 adenocarcinomas of mice. Roussy and Craciun (19, 20), using a histological procedure, attempted to describe the distribution of this material in the Jensen sarcoma and the Rous sarcoma. They distinguished between a "reversible" and "irreversible" form, and claimed to discern five separate zones in the Jensen tumor. Goldfeder (12) made numerous analyses of implanted tumors of mice and chickens, and ascertained that the glycogen content varied inversely with the tumor size. In the Rous sarcoma she found that the periphery of the tumor contained about three times the amount detected in the necrotic center. Feeding glucose or giving it subcutaneously caused higher glycogen values in the tumors but was accompanied by more active tumor growth and an earlier decline of the host. Haendel and Malet (13) found less histological evidence of glycogen in the treated skin and livers of tarred mice than in controls. Babes and Pantzu-Lazarescu (1) found no granules of glycogen in undifferentiated cervical carcinomas but considerable quantities in the partially differentiated forms. Since normal mucous membrane contains this material, it was considered evidence of the degree of differentiation. Faroy (9) supports Brault's contention that glycogen concentration increases with the malignancy of the tumor, and thinks that the determination of glycogen in a given tumor might be of prognostic value. Petrowa (17) examined 22 myomas of the uterus by the histologic method and found considerably more glycogen in the myomas than in the uterine muscle. Lustig and Wachtel (15) found that of many substances mixed with minced tumor inoculum, glycogen was the only substance which inhibited the usual development of tumors.

Several references dealing with the use of glycogen-forming sugars in tumor-bearing animals are of interest. Cori and Cori (7), Goldfeder (12), and Schott, Samuels, and Ball (21), cited above, observed more glycogen in the tumors of animals fed glucose. Parfentjev *et al.* (16) found no effect on the growth rate of sarcoma 180 from glucose or arabinose injected subcutaneously at a distance from the tumor site. Vannfält (23) reported a significantly longer life and fewer metastases in a large series of tarred mice to which a 50 per cent glucose solution was available for drinking. It seems, therefore, that information on the significance of glycogen and the influence of carbohydrates on tumor growth is still problematical.

MATERIALS AND METHODS

Male rats were inoculated with tumor fragments by trocar and one to two weeks later a portion of each group was hypophysectomized. In experiments I to III, inclusive, all were fed twice daily by stomach tube a mixture of 200 gm. of powdered milk, 200 gm. of glucose, 220 ml. water, and 5 Abbott ABD capsules,

* This investigation was aided by a grant from The International Cancer Research Foundation (1938). Technical assistance was received from the National Youth Administration.

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in amounts sufficient to maintain body weight at a constant level. All received subcutaneously 0.1 ml. of adrenal cortex extract daily. In experiment IV, a stock diet was available to the animals ad libitum. Tumors were measured at daily to twice weekly intervals and the growth curve plotted as the best fit straight line. The slope was then estimated.

Tumors were removed under amytal anesthesia at a stipulated time after inoculation or after having reached a certain size, roughly bisected, and a thin slice from one cut surface reserved for histological study by Best's carmine stain after fixation in absolute ethyl alcohol. The remaining tissue was divided into two samples for chemical assay: a small peripheral slice of viable tissue by a micro method, and the remainder including the necrotic center by the standard technic of Good, Kramer, and Somogyi (11) using the micro sugar reagents of Shaffer and Somogyi. The hydrolyzates were subjected to yeast fermentation in every case to exclude errors due to nonfermentable reducing substances.

Completeness of pituitary ablation was checked on the formalin-fixed heads of the animals by removing the superior part of the skull, retracting the brain upwards and back, and examining the sella by direct vision or with the aid of a lens.

The following special conditions apply: Experiments I and II—tumors were removed for analysis on a given day after inoculation. Experiment III—the tumors were taken for analysis when they had attained a given size. Experiment IV—tumors from intact animals on a stock diet were selected from fast and slow-growing transplants or picked at random at regular intervals.

RESULTS

The data in Table I reveal an inverse correlation between the glycogen content of the tumors and their size and rate of growth. The chance of occurrence from random sampling is indicated and in general this is less than 5 per cent; in some instances it approached 1 per cent. In Table II detailed values are presented for experiment III. Of special interest are the glycogen values from the peripheral (most viable) region and for the tumor as a whole (including all central necrosis). A high degree of positive correlation exists ($r = .82$).

An attempt at correlation of histological and chemical determinations of glycogen is given in Table III for 32 tumors. The results are disappointing for the microscopist. Apparently a large proportion of the glycogen must be distributed in solution throughout the tissue. Only in those tumors which showed the largest amounts of glycogen granules is there any significant correlation with the chemical analyses.

TABLE I: CORRELATION COEFFICIENTS (r) BETWEEN GLYCOGEN CONTENT AND SIZE AND RATE OF GROWTH OF TUMORS

	Experiment I	Experiment II	Experiment III	Experiment IV
r_{01} (Glycogen and tumor size)	$-.43 \pm .12$	$-.59 \pm .14$	$-.33 \pm .09$	$-.80 \pm .07$
r_{02} (Glycogen and growth rate)	.....	$-.68 \pm .11$	$-.34 \pm .09$	$-.60 \pm .11$
$r_{02.3}$ (Glycogen and growth rate, excluding the influence of hypophysectomy)	.....	$-.61 \pm .14$	$-.32 \pm .08$	.....
r_{12} (Size and growth rate)	.....	$+.92 \pm .03$	$+.60 \pm .09$	$+.69 \pm .10$
Number of animals	20	11	46	15
Coefficients for occurrence from random sampling				
5 per cent chance	.44	.60	.29	.51
1 per cent chance	.56	.73	.38	.64

x_0 = glycogen percentage in total tumor.
x_1 = size (weight).
x_2 = growth rate (mm. mean diameter per day).
x_3 = hypophysectomy (expressed as unity for hypophysectomized rats and zero for intact animals).

TABLE II: DATA FROM EXPERIMENT III

	Controls (32)		Hypophysectomized (14)		Difference of means
	Range	Mean	Range	Mean	
Tumor weight in gm	3.8 — 16.2	$6.57 \pm .33$	2.7 — 6.2	$4.57 \pm .16$	$1.99 \pm .37$
Growth rate, mm. per day	.34 — 1.31	$.69 \pm .028$	.30 — .84	$.59 \pm .03$	$.10 \pm .04$
Glycogen in total tumor, per cent	.013 — .114	$.062 \pm .003$	.025 — .114	$.071 \pm .005$	$.009 \pm .006$
Glycogen in peripheral slice, per cent	.005 — .121	$.063 \pm .003$	.027 — .114	$.069 \pm .005$	$.006 \pm .006$

DISCUSSION

The increased glycogen concentration in the slow-growing tumors may be either a result or a cause of slow growth. The first assumption seems more probable. Since slow-growing tumors, whether produced by hypophysectomy or other causes, require less energy, the increase in glycogen values is probably a matter of accumulation incident to decreased utilization. Since glycogen accumulates when tumor utilization is probably decreased, it follows that glycogenesis is a more constant process than utilization, and is not influenced to an equal extent by the same factors. Glycogenesis appears to be primarily dependent on the supply of materials from which glycogen can be formed since the concentrations within both fast- and slow-growing tumors are increased by feeding large amounts of carbohydrates (21).

Even though relatively large areas of necrosis were present centrally and absent peripherally, glycogen was rather uniformly distributed throughout the tumor. It would seem, then, that the enzyme system involved is not dependent on the immediate presence of viable cells, or that greater diffusion of glycogen has occurred than would ordinarily be supposed. Our findings in this respect differ sharply from those of Goldfeder (12). It seems possible that the difficulty to be encountered in separating the peripheral part of the Rous sarcoma from the adjacent muscles might reasonably account for this difference.

TABLE III: CORRELATION OF HISTOLOGICAL AND CHEMICAL DETERMINATIONS OF GLYCOGEN IN 32 TUMORS

Histological appraisal	Glycogen percentage (chemical)								
	.00-.02	.02-.03	.04-.05	.05-.06	.06-.07	.07-.08	.08-.09	.09-.10	.11-.12
0	..	1	1	..	..	..	..	..	..
1+	2	2	1	2	..	1	..	..	..
2+	2	1	2	2	1	2	..	1	..
3+	..	..	2	..	..	..	5	..	..
4+	..	..	..	..	..	..	1	1	2

SUMMARY AND CONCLUSIONS

1. The glycogen content of Walker 256 tumor is inversely correlated with its size and rate of growth.

2. The increased glycogen concentrations which have been observed in the tumors of hypophysectomized rats are due to slower growth, since elimination of the influence of hypophysectomy did not significantly alter the correlation between glycogen content and rate of tumor growth.

3. Necrotic areas of tumor contain glycogen in amounts comparable with those found in proliferating areas.

4. Little correlation was found between the amount of material histologically distinguished by its carmine-staining reaction, and polysaccharide which can be separated from tumor tissue by the usual biochemical technic. Only when the concentration of glycogen is relatively high is there a significant increase in glycogen granules in tissue preparations.

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Corrections

See: Lewisohn, R., C. Leuchtenberger, R. Leuchtenberger, D. Laszlo, and K. Bloch. Action of Yeast Extract on Transplanted and Spontaneous Tumors in Mice. Cancer Research, **1**:799-806. 1941.

The following corrections of errors in the manuscript are published at the request of the authors:

1. P. 799, column 2, line 14: For "(No. 3, Table I)" substitute "(No. 2, Table I)."

2. P. 799, footnote 2: For "No. 3" substitute "No. 2"; for "0.01γ" substitute "0.1γ."

3. P. 800, column 1, paragraph 2, line 9: Insert "in the filtrate" after "it was present."

4. P. 804, column 2, last line: Delete "nor" and insert "and is."

5. P. 806, column 1, line 1: Insert "not" after "The active material is."

6. P. 800, Table I, substitute the following:

Table I: Summary of Tests of Fractions of Yeast Extract

Extract No.	Preparation	Dose in mgm.	Per cent of solids compared with yeast extract	Total number of animals	Complete * regressions
1.	Ethanol precipitate after dialysis	3	28	37	9
2.	Lead filtrate from No. 1	3	22	27	9
3.	Silver filtrate from No. 2	2	11	23	7
4.	Lead filtrate from undialized yeast extract	4	25	19	5
5.	Lead precipitate from undialized yeast extract	4	37	7	0
6.	Silver filtrate from No. 4	4.5 1.5	16	16†	5
7.	Silver precipitate from No. 4	1	11	8	0
8.	Ethanol-insoluble barium salts from No. 6	0.9	3.2	11	4
9.	Phosphotungstate from No. 6	0.7	2.5	9	3
10.	No. 1 after nitrous acid treatment	7.5	..	7	2
11.	No. 1 after treatment with N/2HCL	4.5	..	9	0

* Temporary regressions with subsequent recurrences are not included in these statistics.
† 8 mice received 4.5 mgm., and 8 received 1.5 mgm. in single doses.

Abstracts

Reports of Experimental Research

Carcinogenic Compounds

ANDERVONT, H. B., and J. E. EDWARDS. [Nat. Cancer Inst., Bethesda, Md.] HEPATIC CHANGES AND SUBCUTANEOUS AND PULMONARY TUMORS INDUCED BY SUBCUTANEOUS INJECTION OF 3,4,5,6-DIBENZCARBAZOLE. J. Nat. Cancer Inst., 2:139-149. 1941.

Single subcutaneous injections of 0.2 mgm. quantities of 3,4,5,6-dibenzcarbazole dissolved in lard, sesame oil, or olive oil were made into the right axillary region of mice from strains C3H, C, and A. Tumors produced at the site of injection and livers were examined grossly and microscopically.

Male mice of these strains were found generally to be less susceptible to liver damage than female mice. Females of all 3 strains showed liver damage when sesame oil was used but strain A mice showed liver changes only when lard was used. Castration of the male strain A mice increased the percentage of cases of liver damage from a control figure of 14.3% to 47% in the castrates. Thirty-eight per cent of castrated males receiving stilbestrol developed liver damage.

Lard solutions were less active in producing subcutaneous fibrosarcomas than solutions of the carcinogen in sesame oil. Male mice are more susceptible to subcutaneous tumor production by lard solutions of 3,4,5,6-dibenzcarbazole than are the females. Pulmonary tumors were produced in strain A female mice as early as the 10th week. An incidence of 66 to 70% of pulmonary tumors occurred. The type of solvent did not materially vary the occurrence of these tumors.

Regardless of the strain or hybridization, the sex susceptibility to liver damage due to 2-amino-4-azotoluene in 10 mgm. doses per month for a period of 10 months is demonstrable. It is suggested that the rather high urinary protein excretion in male mice may enable them to excrete more of the carcinogen. The influence of sex was more pronounced in determining the degree of susceptibility to induced hepatic changes than to induced subcutaneous growths.—R. C. R.

EDWARDS, J. E. [Nat. Cancer Inst., Bethesda, Md.] HEPATOMAS IN MICE INDUCED WITH CARBON TETRACHLORIDE. J. Nat. Cancer Inst., 2:197-199. 1941.

Of 143 male C3H mice fed 23 or more 0.1 cc. feedings of a 40% olive oil solution of carbon tetrachloride given 2 to 3 times a week, 126 or 88.1% developed hepatomas. In a similar experiment with 54 mixed sex strain A mice, 100% of the mice developed hepatomas. No hepatomas were found in 17 C3H controls. A group of mice fed olive oil without carbon tetrachloride showed an incidence of hepatoma in 4.3% of the cases. Whether the tumors themselves are directly due to the effect of the carbon tetrachloride or to the hepatic damage awaits further study.—R. C. R.

EDWARDS, J. E., and J. White. [Nat. Cancer Inst., Bethesda, Md.] PATHOLOGIC CHANGES, WITH SPECIAL REFERENCE TO PIGMENTATION AND CLASSIFICATION OF HEPATIC TUMORS IN RATS FED *p*-DIMETHYLAMINOAZOBENZENE (BUTTER YELLOW). J. Nat. Cancer Inst., 2:157-183. 1941.

One hundred and six 1-month-old rats of the Osborne-Mendel strain were fed basal diets and butter yellow supplements. All animals were autopsied between 2 and 52 weeks of butter yellow feeding and microscopic sections were obtained. Sixty-six of these animals developed hepatomas and 20% of these tumors metastasized.

The gross pathology of the liver, peritoneum, kidney, spleen, lungs, lymph nodes, and gastrointestinal tracts of these rats is described. Microscopic examination of the hepatomas divides them into two general classes, the hepatoma and the adenocarcinoma. The hepatoma was subdivided again into two subclasses; namely, a type which was well differentiated and at times encapsulated resembling normal liver parenchyma, and a second type composed of less differentiated cells but still maintaining the general structural pattern of liver tissue. Concerning the origin of the adenocarcinoma, the authors consider functions and anatomic relationships of hepatic and bile duct cells. It is concluded that the adenocarcinoma probably originated from the hepatic parenchymal cells.

The occurrence of bone was noted in several of the hepatic tumors. It was considered probably to be due to the stimulation of metaplasia of fibroblasts to osteoblasts by the regenerating transitional epithelium.

There were three types of pigment observed in these animals. They were: 1. an iron-containing, granular, brown pigment found in the liver, spleen, and lymph nodes; 2. one found in the tubular cells and macrophages of the renal cortex was brown and granular and gave no test for iron; and 3. one deposited in the liver and lymph nodes as a canary yellow material. It was lipoidal in nature. The tumor cells themselves were not observed to contain pigment nor glycogen.—R. C. R.

SHEAR, M. J., J. LEITER, and A. PERRAULT. [Nat. Cancer Inst., Bethesda, Md.] STUDIES IN CARCINOGENESIS. XV. COMPOUNDS RELATED TO 20-METHYLCHOLANTHRENE. J. Nat. Cancer Inst., 2:99-112. 1941.

Data obtained following the injection of 22 compounds related structurally to 20-methylcholanthrene are reported. The derivatives were classified in 6 groups. These groups were: 1. alkyl derivatives, 2. oxygen-containing derivatives, 3. chloro, bromo, and cyano compounds, 4. anthracene derivatives, 5. bile acids and choleic acids, and 6. a miscellaneous group. Carcinogenic potency for mice was determined by gross and microscopic observations on tissues at the site of injection and by the occurrence of multiple tumors of the lungs. A total of 475 mice of inbred strains, chiefly strain A, was used. Crystalline derivatives were moistened in glycerol and injected. When

too small a quantity of material was available lard was used to dissolve the crystals. Lard and glycerol were used in preference to sesame oil because of the carcinogenic action of certain products of this oil. Carcinogenic potency for compounds in group 1, reported in order of decreasing activity, was: methylcholanthrene, cholanthrene, 15,20-dimethyl-, 20-ethyl-, 16,20-dimethyl-, 20-*iso*-propyl-, and 20-*t*-butylcholanthrene. Two of the seven oxygen-containing derivatives displayed carcinogenic activity. These were 15-hydroxy- and 15-keto-20-methylcholanthrene. Group 3 showed no carcinogenic activity. The only anthracene derivative shown to be carcinogenic was 1,2-cyclopenteno-5,10-aceanthrene. This conforms to the fact that the angular benzene ring of 1,2-benzanthracene is not essential for activity. Its presence however confers a high order of activity on anthracene derivatives. Desoxycholic acid of group 5 was found to be toxic when injected in glycerol and in water. Small amounts of crystals of the acid had no such effect. This acid was shown to be noncarcinogenic. The compounds 1,2,5,6-dibenzanthracene-choleic acid and 20-methylcholanthrene-choleic acid both showed carcinogenic activity. No tumors were produced with choleic acid or acenaphthene-choleic acid. Miscellaneous other compounds found to be carcinogenic were 6-methylchrysene and 4,9-dimethyl-1-2-benzanthracene.—R. C. R.

SPENCER, R. R., and M. B. MELROY. [Nat. Cancer Inst., Bethesda, Md.] EFFECT OF CARCINOGENS ON SMALL ORGANISMS. III. CELL-DIVISION RATE AND POPULATION LEVELS OF METHYLCHOLANTHRENE-ADAPTED PARAMECIA. J. Nat. Cancer Inst., 2:185-191. 1941.

Paramecium multimicronucleatum has been shown to survive partial starvation for longer periods of time after prolonged exposure to carcinogens than do normal paramecia of the same species.

The population curve of these microorganisms after the 44th transfer in methylcholanthrene was seen to reach a maximum of nearly 600 organisms in 15 days whereas the normal organisms reached a level of 165 in 2 days and then declined. This phenomenon was not observed until the above number of transfers was reached

Several amino acids were found to stimulate cell division in both the controls and adapted paramecia. Calcium pantothenate markedly increased the cell populations in both controls and methylcholanthrene-adapted organisms. If *Staphylococcus albus* was added to the organisms as food, increases occurred in the population curve in both controls and methylcholanthrene-adapted organisms. Fluorescein-adapted organisms failed to show as marked a change as the normals after staphylococcus feeding. Testosterone propionate was found to be markedly toxic to both the adapted and the normal controls. A mild inhibitory effect was caused in both types by colchicine. This potentiality for prolonged survival is retained in considerable degree even after the species is removed from the methylcholanthrene for 5 to 10 transfers. No structural changes have been noted to date in the methylcholanthrene-adapted group.

These findings seem to throw some light on the implausibility of the tissue tension theory of cancer. In these tests, a free-living organism relieved of all growth-restraining influences seemed to be endowed with more than the ancestral power of cell division and survival when the species was exposed to a blastogenic hydrocarbon for many generations. These carcinogens seem actually to enhance certain vital functions rather than decrease them.—R. C. R.

STEWART, H. L., and E. LORENZ. [Nat. Cancer Inst., Bethesda, Md.] INDUCTION OF ADENOCARCINOMA OF THE PYLORIC STOMACH IN MICE BY METHYLCHOLANTHRENE. J. Nat. Cancer Inst., 2:193-196. 1941.

Four cases of induced adenocarcinoma of the stomach in C3H mice are reported. This strain of mice has never developed spontaneous adenocarcinoma of the stomach in the author's laboratory. The tumors were produced by direct injection into the wall of the pyloric chamber of the stomach of C3H mice. The material injected was a horse serum dispersion of methylcholanthrene. It was injected in amounts varying between 0.03 to 0.05 cc. This represented about 0.6 to 1.0 mgm. of the methylcholanthrene. If 100% pellets or mineral oil solutions of methylcholanthrene were used, the tumors produced were mixed with sarcoma. Adenoacanthoma of the stomach has also occurred in this group of mice.—R. C. R.

Viruses

BEARD, D., D. G. SHARP, A. R. TAYLOR, and J. W. BEARD. [Duke Univ. Sch. of Med., Durham, N. C.] VIRUS DISTRIBUTION IN SOLUBLE AND INSOLUBLE PHASES OF NEUTRALIZED PAPILLOMA-VIRUS PROTEIN. Proc. Soc. Exper. Biol. & Med., 47:502-504. 1941.

Direct evidence is provided for the existence of both soluble and insoluble phases of neutralized virus and for the dissociation of both with dilution when the 2 phases are separated. An estimate is afforded of the distribution of virus between the 2 phases separated in this way by centrifugation. This distribution however can be considered only for the special region of serum-virus amounts employed. The reduction in free virus in the supernatant fluid could not have been due to sedimentation of free virus since it could not be found in the pellet suspension. The possibility of a shift in chemical equilibrium is suggested to explain this phenomenon.—M. B.

Immunology

HOCH-LIGETI, C. [The Roy. Cancer Hosp. (Free), London] STUDIES ON THE EFFECT OF CARCINOGENIC HYDROCARBONS ON IMMUNITY REACTIONS. Brit. J. Exper. Path., 22:233-240. 1941.

Rabbits which had received colloidal polycyclic hydrocarbons (methylcholanthrene, 1,2,5,6-dibenzanthracene, 1,2-benzanthracene, phenanthrene) *sub cutem* during one year were immunized with horse serum. All the animals developed a strong immunity. There was no difference between the amounts of precipitin produced in the blood of the animals treated with carcinogenic and noncarcinogenic hydrocarbons.

Rabbits were immunized with horse serum, and then received injections of colloidal hydrocarbons *sub cutem*. Forty-eight hours after injection of 1,2-benzanthracene or anthracene the optimal proportion was unchanged or slightly lowered; in 7 out of 9 animals receiving methyl-

cholanthrene a sudden drop occurred and the time of precipitation was generally prolonged. Estimation of the nitrogen precipitated showed a smaller difference between the effects of the two classes of hydrocarbons.—E. L. K.

RODRIGUES, A. [Inst. Portugues de Oncologia, Lisbon] O SISTEMA LINFÁTICO E O PROBLEMA DO CANCRO. [THE LYMPHATIC SYSTEM AND THE PROBLEM OF CANCER.] Arq. de pat., 12:451-489. 1940.

After some considerations of the influence of the lymphatic system on the genesis and evolution of cancer, the author describes experiments carried out on the rat in order to prove the defensive role of the lymphatic nodes against the dissemination of the tumor. The lateral-aortic nodes of 16 rats were removed and a spontaneous sarcoma of the same animal was inoculated in the left posterior limb. Some days later 81% of the operated animals showed intra-abdominal metastasis whereas in the controls no metastasis developed. At the same time the operated animals were more sensitive to the tumor which had a shorter evolution, was larger and more invasive of the neighboring tissues. In order to see if the anticancerous power of the nodes depended on the presence of this tissue or was in proportion to its degree of development half of the lateral-aortic nodes were left intact in some animals, and the results were the same as in the experiment mentioned above. Other experiments showed that the defensive power of the nodes is manifest only when they are connected with the lymphatic vessels.—M. D-R.

Transplantation

ANDERVONT, H. B., and M. B. SHIMKIN. [Nat. Cancer Inst., Bethesda, Md.] INTRAPULMONARY TRANSPLANTATION OF THE ADENOMATOUS GASTRIC LESION OF STRAIN I MICE. J. Nat. Cancer Inst., 2:151-155. 1941.

Strain I mice develop spontaneous adenomatous lesions in the glandular region of the stomach. This is found in most of these mice living to be 8 months old or more. It is more pronounced in animals eating roughage. It appears to be a recessive hereditary character. Histologically, the lesions appear malignant, but grossly they are benign. They do not appear in F_1 hybrids of strain I mice.

An attempt to transplant this lesion was made. It was minced and suspended in saline and injected intravenously into strain I and strain I hybrid mice. Pulmonary takes similar to the original lesion were found in a large percentage of cases. Strain I hybrids were found to be less susceptible than the pure strain I mice. These pulmonary tumors were found not to be subcutaneously transplantable. Control mice injected with normal stomach mucosa were negative in most cases after 6 months' time. Minces of liver and kidney injected in a similar manner into strain C mice produced no pulmonary lesions.

These results tend to indicate that the hyperplastic mucosa of strain I mice possesses a greater capacity for autonomous growth than does normal stomach mucosa, liver, or kidney.—R. C. R.

BREEDIS, C., T. ROBERTSON, R. S. OSENKOP, and J. FURTH. [Cornell Univ. Med. Coll., New York, N. Y.] OBSERVATIONS ON THE CHARACTER OF CHANGES OCCURRING IN THE COURSE OF TRANSPLANTATION OF TWO STRAINS OF LUNG TUMORS IN MICE. Cancer Research, 2: 116-124. 1942.

Two transmissible tumors originated in adenomas of the lung of mice. One grew in the subcutaneous tissues and assumed a sarcoma-like appearance in the course of the first two subpassages. Histological studies suggest that this change is due to a modification in appearance of the tumor cells. This modification is fixed and is not altered by different environment or conditions. The second strain could at first be transmitted only by the intravenous route. It is characterized by affinity for lungs and by a very long incubation period which has not changed in the course of four successive passages during which the neoplastic cells retained their adenoma carcinoma-like character. It is unchanged by growth in the subcutaneous tissues.—Authors' abstract.

NAGAYO, M. [Labs. of the Japanese Foundation for Cancer Research, Tokyo] ON HETEROPLASTIC TRANSPLANTATION OF MOUSE SARCOMA. Gann, 35:233-246. 1941.

A sarcoma produced by daily injection of fructose into a mouse for 296 days was successfully transplanted to rats. The tumor was first grown for 38 generations in mice, during which time it changed histologically. The tumor originally contained characteristic slender spindle cells with many collagenous fibers and occasional hyalinized areas, but after the 30th transplant the predominant cells were short, closely packed spindle and polymorphous cells. The transplantation from mice to rats was successful more than a dozen times, and the tumors resulting were identical with the mouse tumors. After growing in rats for several generations the tumors could be transplanted back to mice without any evidence of histological change. Attempts to transplant the tumor to rabbits, guinea pigs, and chickens failed. Desiccated tumor tissue of Berkefeld filtrates failed to induce tumors in mice. The incidence of takes was 80 to 100% in mice, and about 40% in rats. Careful histological studies at short intervals after transplantation of small bits of tumor from mice to rats revealed that the rat tumors arose from the cells of the transplanted tumors. In contrast to mice, which showed no regression once the tumor started to grow, 13 out of 141 rat tumors showed regression. It was observed that preliminary injections of mouse blood or spleen emulsion to rats 3 to 16 days before transplantation of the mouse tumor resulted in a lower percentage of takes. According to the author this represents an acquired immunity.—P. P. C.

Clinical and Pathological Reports

Radiation—Diagnosis and Therapy

CUTLER, M. [Chicago Tumor Inst., Chicago, Ill.] CONCENTRATION METHOD OF RADIOTHERAPY FOR CANCER OF THE MOUTH, PHARYNX AND LARYNX. J. A. M. A. 117:1607-1610. 1941.

A new principle and technic of external irradiation gives a more pronounced effect on the more radioresistant forms of cancer of the mouth, pharynx, and larynx than the methods of external irradiation now in use. This technic has been called the method of concentration. It can be applied daily and continuously or in two cycles interrupted by a rest period of 11 to 15 days. Since this method has been used but 3½ years nothing can be said of the permanence of the results, but certain carci-

nomas which failed to respond to all other methods of external irradiation have showed decided retrogression and in many instances have disappeared completely.—H. G. W.

SHARP, G. S. [Pasadena, Calif.] THE RADIATION TREATMENT OF CANCER OF THE RECTUM; TECHNIQUE FOR RADIUM NEEDLES THROUGH PERINEAL STAB WOUNDS. Am. J. Roentgenol., 46:207-218. 1941.

Forty-two consecutive patients with carcinoma of the rectum were treated by radiation. Twenty-eight of these were treated prior to 1937 and have now been followed at least 4 years. Treatment was palliative in 15 while cure was attempted in 13. The technic of treatment is described in some detail. In brief, it consisted of external roentgen irradiation in doses of 1,200 r to 2,000 r delivered to each of 4 pelvic portals and followed by implantation of radon seeds in the tumor. In many of the later cases perirectal radium needles were also used. Seven of the 13 patients were living, or dead without evidence of carcinoma at autopsy, 4 to 8 years after treatment was begun; two were living with disease at 5 and 8 years respectively and the remaining 4 were dead of disease. The 15 patients in the palliative group died in 1 to 30 months except for one who was living and well at the end of 4 years. The author does not state the indications for using radiation therapy in preference to surgery, but states that 7 of his cases were considered operable.—C. E. D.

SPENCER, J. [Huntington Memorial Hosp., Boston, Mass.] RADIATION TREATMENT OF HEMANGIOMA. Am. J. Roentgenol., 46:220-223. 1941.

The results of radiation treatment of 137 cases of hemangioma in infants are discussed. Although there was considerable variation in the technic of irradiation, the cases are divided into 3 main groups: 1. Patients treated between 1920 and 1930 with large doses of radium applied both in contact with the lesion and at a distance showed fair results but scars resulted and one patient developed malignancy in the scar 15 years after treatment. 2. Patients treated with small doses of filtered radon at 1 cm. distance showed complete regression in all of 21 cases followed. 3. Patients treated with suberythema doses of 200 kv. roentgen rays likewise showed excellent results in the brief follow-up period of 4 months to 2 years. Treatment of hemangioma is not advised before the age of 6 months since these lesions may regress spontaneously.—C. E. D.

TENZEL, W. V. [Montefiore Hosp., New York, N. Y.] RADIATION THERAPY IN CARCINOMA OF THE LUNG. J. A. M. A., 117:1778-1782. 1941.

An analysis of 192 treated and untreated cases of carcinoma of the lung which have been examined postmortem at the Montefiore Hospital from 1921 to 1939, demonstrates the value of radiation therapy in prolonging life, the survival time of irradiated patients being 5 months longer, and symptomatic relief being obtained in 45%. In one-third of the cases of epidermoid cancer there were no distant metastases, while in adenocarcinoma and anaplastic cancer there were distant metastases in 92%. On the other hand, abscess was present in 50% of the cases of epidermoid cancer as against 9% in the adenocarcinoma group and 18% of the anaplastic group. Thirty-four per cent of the patients had skeletal metastases, only 3% being osteoplastic.—H. G. W.

Skin and Subcutaneous Tissues

BERESTON, E. S., and C. NEY. [Montefiore Hosp. for Chronic Diseases, New York, N. Y.] SQUAMOUS CELL CARCINOMA ARISING IN A CHRONIC OSTEOMYELITIC SINUS TRACT WITH METASTASIS. Arch. Surg., 43:257-268. 1941.

Two such cases are reported. In the first, metastases were found in the lung; in the second, they were found in the pelvic bones.—G. De B.

FLEMING, R. M., and P. R. REZEK. [Jackson Memorial Hosp., Miami, Fla.] SARCOMA DEVELOPING IN AN OLD BURN SCAR. Am. J. Surg., 54:457-465. 1941.

Carcinomas are not infrequent in old burn scars, but sarcomas are rare. The authors report the case of a sarcoma arising in a burn scar of 49 years' duration, which produced death by metastases.—H. G. W.

HEROLD, W. C., W. H. KAUFMAN, and D. C. SMITH. [Univ. of Virginia, Charlottesville, Va.] ACANTHOSIS NIGRICANS. Arch. Dermat. & Syph., 44:789-797. 1941.

A case of acanthosis nigricans of the malignant type associated with gastric carcinoma in a 17-year-old girl is reported. No abnormality of the adrenals was demonstrable.—H. G. W.

PACK, G. T., and D. A. SUNDERLAND. [Memorial Hosp., New York, N. Y.] NAEVUS UNIUS LATERIS. Arch. Surg., 43:341-375. 1941.

The authors describe 4 cases of this entity observed at the Memorial Hospital, and analyze 156 cases collected from the literature.

Almost half the lesions appear at birth or shortly afterward; the remainder appear in early life. Over two-thirds of the nevi show pigmentation. Microscopically this lesion consists of hyperkeratosis, distortion of the epidermis, and elongation of the papillae. The authors believe that surgical excision, usually in stages, is the best treatment. The cause of this condition is still unknown. The distribution of the nevi does not follow precisely central or peripheral nerves. When the lesions become malignant, they take the form of epidermoid carcinoma and not of melanoma.—G. De B.

SYMMERS, D. [Bellevue Hosp. Labs. of Pathology, New York, N. Y.] KAPOSI'S DISEASE. Arch. Path., 32:764-786. 1941.

A study of 8 cases among 25 observed of Kaposi's disease, "multiple idiopathic hemorrhagic and pigmented sarcoma," which occurs chiefly in Jews and Italians over 40 years of age, and predominantly in males. Only 3 cases, including 1 in this report, have been observed in Negroes. The unit of growth is the fibroblast which lies in the papillary layer of the derma, and from the subepithelial fibroblasts in the mucosa. The fibroblast determines the growth behavior of each of the phases by which Kaposi's disease may be recognized, at times producing young argentophilic collagenous fibrils which may aid in the process of healing, or again maintaining a low capacity of growth for years, or assuming malignant properties of a sarcoma. Histologic observation shows

that from the outset two opposing factors are operative; one tending to bring about replacement of the nodules by production of collagenous fibrils, the other tending to maintain the capacity of the nodule to grow, but at an extremely low rate. The growths in Kaposi's disease are histologically malignant but clinically benign. The process of retrogression and spontaneous healing is probably initiated by hemorrhages followed by injury to the fibroblasts followed by connective tissue replacement.—H. G. W.

Nervous System

GARDNER, W. J., and O. TURNER. CRANIAL CHORDOMAS. Arch. Surg., 42:411-425. 1941.

Three cases are added to the literature.—G. De B.

KERNOHAN, J. W. [Mayo Clinic, Rochester, Minn.] TUMORS OF THE SPINAL CORD. Arch. Path., 32:843-883. 1941.

A general review of the literature on the histogenesis, microscopic appearance, distribution, and frequency of tumors of the spinal cord and its membranes.—H. G. W.

PETERSON, H. O., and A. B. BAKER. [Univ. of Minnesota, Minneapolis, Minn.] DIFFICULTIES IN DIFFERENTIATING MIDBRAIN LESIONS FROM CEREBELLAR LESIONS. Am. J. Roentgenol., 46:37-51. 1941.

The authors disclaim ability to localize brain tumors radiographically with the high degree of accuracy sometimes claimed in the literature. Ten case histories together with excellent roentgenograms are presented and discussed in an attempt to illustrate the difficulties of diagnosis and localization of tumors of the midbrain and cerebellum. If the aqueduct and fourth ventricle are not visualized by ventriculography, localization is uncertain. Helpful technical procedures are described.—C. E. D.

Breast

BEACH, A. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] THE EFFECTS OF ROENTGEN-RAY DOSAGE IN CARCINOMA OF THE BREAST. Am. J. Roentgenol., 46:89-95. 1941.

In all of the author's cases of carcinoma of the breast treated by preoperative irradiation histological evidence of persistent carcinoma was found. The 5 cases which received the largest doses—often in excess of 8,000 r—are described in detail with photomicrographs of the radiation changes and of the residual tumor tissue. No conclusions are drawn concerning the clinical value of preoperative irradiation.—C. E. D.

GUENIN, P. [Dept. Surgery, Faculty of Med., Paris] EL PRONOSTICO DEL CANCER DEL SENO. [PROGNOSIS OF MAMMARY GLAND CANCER.] Rev. de med. y cir. Habana, 56:185-202. 1941.

The author gives a detailed account of his technic of pre- and postoperative radiation based on an extensive research on the anatomy and physiology of the lymphatics of the mammary glands. He states that following his method the late prognosis of the disease is improved.—M. D-R.

OWENS, F. M., JR., and W. E. ADAMS. [Univ. of Chicago, Chicago, Ill.] GIANT INTRACANALICULAR FIBROADENOMA OF THE BREAST. Arch. Surg., 43:588-598. 1941.

The history of this breast tumor is briefly given, and a case is added to the literature.—G. De B.

POHLE, E. A. [Univ. of Wisconsin Med. Sch., Madison, Wis.] STERILIZATION OF THE OVARIES BY ROENTGEN RAYS IN THE TREATMENT OF DISTANT METASTASES FROM PRIMARY CARCINOMA OF THE BREAST. Am. J. Surg., 54:490-493. 1941.

Two illustrative cases of palliation of metastases in carcinoma of the breast following sterilization by x-rays are reported, and the routine sterilization of all women who have metastatic carcinoma is recommended.—H. G. W.

SAPHIR, O., and M. L. PARKER. [Michael Reese Hosp., Chicago, Ill.] METASTASES OF PRIMARY CARCINOMA OF THE BREAST. Arch. Surg., 42:1003-1018. 1941.

In 43 patients who came to autopsy with primary carcinoma of the breast, the authors found that metastases involved the adrenals 19 times, the spleen 10 times, and the ovaries 7 times. They regard as indicative of a high degree of malignancy the presence of tumor cells, regardless of the type of carcinoma, separated from the basic structure of the primary tumor. Most of the tumors which produced metastases to these three organs showed an infiltration of isolated carcinoma cells outside the primary site.—G. De B.

ZONDEK, B. [Hebrew Univ., Jerusalem, Palestine] INCREASED EXCRETION OF GONADOTROPIC HORMONE IN A PREGNANT WOMAN WITH MAMMARY CARCINOMA. J. Clin. Endocrinol., 1:782-783. 1941.

A brief review is given of previous claims that follicle-stimulating hormone in the urine is increased in patients with carcinoma, especially in persons with cancer of primary and various secondary sex organs. Six assays are reported for one pregnant woman who had a large mammary carcinoma with metastasis to the axilla. A high titer (>300,000 M.U./liter, assayed apparently from urine collected on one day) was observed at the 8th month of pregnancy, somewhat lower titers (100,000 M.U./liter) after radical mastectomy, and still lower values (1,000 M.U./liter) after delivery.—J. B. H.

Female Genital Tract

CACCIA, J. P., and J. C. VILLANUEVA. [Hosp. Rosario, Buenos Aires] FIBROANGIOMA DEL LABIO MENOR. [FIBROANGIOMA OF LABIUM MINUS.] Publ. Médicas, 6:205-210. 1941.

The author reviews the literature on the subject before describing his case. According to statistics quoted by the author, out of 14,000 cases of tumors studied in the Hospital Rowson in 14 years, 34 were primary tumors of the vulva (0.20%) of which 67% were malignant. In relation to tumors of the genital system in general the proportion, according to Ahumada, is 2.03%. The author points out the necessity of early and radical treatment, always surgical, due to the sarcomatous degeneration and ulceration of the skin that, according to Leonhard, takes place in 20% of the cases.—M. D-R.

FUNK-BRANTANO, P. [Faculty of Méd., Paris] EVOLUCION DE IDEAS SOBRE EL TRATAMIENTO DEL FIBROMA UTERINO EN LA CLÍNICA DE LA SALPETRIÈRE. [EVOLUTION OF IDEAS CONCERNING THE TREATMENT OF FIBROMAS OF THE UTERUS IN THE SURGICAL CLINIC OF THE SALPETRIÈRE.] Rev. de med. y cir. Habana, 56:144-153. 1941.

Prof. Gosset in the Salpetrière of Paris, after having treated 1,170 cases of uterine fibroma by supravaginal

hysterectomy, published in 1938, together with Charrier, his technic of total hysterectomy for the treatment of the disease. The author, a pupil of Gosset, compares the advantages and disadvantages of both methods and concludes that total hysterectomy is the method of choice. Also surgery would be preferable to radiation in most cases.—M. D-R.

HARDING, W. G., 2ND. [Hornsby District Hosp. and Univ. of Sydney, Sydney, Australia] THE INFLUENCE OF SYPHILIS IN CANCER OF THE CERVIX UTERI. Cancer Research, 2:59-61. 1942.

A comparison of the histologic degree of malignancy, clinical stage when first diagnosed, and the age factor in a series of 227 consecutive cases of cervical carcinoma showed the syphilitic group of 36 cases to have a higher percentage of extensive involvement, a lower percentage of low malignancy (grade 1, Martzloff classification), and an average age of 4 years less than the nonsyphilitic group.—Author's abstract.

SCOTT, G. D., and I. H. SCOTT. [Sullivan, Ind.] EXTRAUTERINE FIBROMYOMAS. Am. J. Surg., 54:391-394. 1941.

Only about 250 cases of extrauterine fibromyomas have been reported, to which the authors add 3: a fibromyoma of the vagina and two in the broad ligament, one of which had undergone sarcomatous degeneration.—H. G. W.

Male Genital Tract

HAMILTON, J. B., and J. B. GILBERT. [Yale Univ. Med. Sch., New Haven, Conn.] STUDIES IN MALIGNANT TUMORS OF THE TESTIS. IV. BILATERAL TESTICULAR CANCER. INCIDENCE, NATURE, AND BEARING UPON MANAGEMENT OF THE PATIENT WITH A SINGLE TESTICULAR CANCER. Cancer Research, 2: 125-129. 1942.

Attempts at exhaustive examination of the literature from 1805 to 1939 resulted in the collection of 144 instances of bilateral testicular tumors. In men with cancer of one testis the likelihood of cancer in the other testis is from several hundred to several thousand times greater than would be expected in chance association.

Abdominally-retained testes are especially prone to cancer in both organs. One in every 8 men with bilateral testicular cancer had abdominally-retained testes, a condition which could be estimated to occur in probably much less than 0.005% of men.

Bilateral tumors occur predominantly in the reproductive years of life, but the first tumor usually occurs later in life than in the unilateral cases. The greater number of both unilateral and bilateral tumors, especially those occurring during the reproductive years, are of unicellular types. Further evidence is advanced to the effect that prenatal factors are significant in the predisposition to testicular cancer.

Management of the case with cancer of one testis must be concerned with the possibility of primary involvement of the other testis. Despite the usual rapidly fatal course in the patient with testicular cancer, a tumor has appeared in the other testis in 15% of those with inguinal testes and 30% of those with abdominal testes. The second testis of a man with a bilateral cryptorchidism and cancer of one testis must be viewed as a site of a potential carcinogenesis.—Authors' abstract.

JORSTAD, L. H. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] CARCINOMA OF THE PENIS. Am. J. Roentgenol., 46:232-235. 1941.

A brief critical review of the literature on carcinoma of the penis is presented. On the basis of these reports and the author's experience with 42 cases, the optimal methods of treatment are discussed. The tumors are generally of a low grade of malignancy. Inguinal metastases are present in over 1/3 of the cases while pelvic metastases are less common. Radiation treatment of the primary tumor or of lymph node metastasis has been disappointing. Amputation of the penis followed by block dissection of the inguinal and femoral regions offers the best chance of cure and does not result in "economic deformity." Radical amputation of the penis is not always indicated and removal of the testicles is seldom justified. Postoperative irradiation is considered illogical when evidence of disease is absent. In the author's series of 42 cases, 75% were living and well 5 years or more after treatment.—C. E. D.

TWOMBLY, G. H., and A. F. HOCKER. [Memorial Hosp., New York, N. Y.] CHORIOEPITHELIOMA IN THE MALE TREATED WITH PREGNANCY SERUM. Surg., Gynec. & Obst., 73:733-739. 1941.

Based on the hypothesis that there may be some substance in the serum of pregnant women which inhibits the growth of chorionic villi which invade and produce emboli in normal women, treatment with pregnancy serum was attempted in a male with a chorioepithelioma with metastases, without benefit. The pituitary gland showed an increase in basophilic cells and the degranulation of the eosinophiles characteristic of pregnancy. The patient excreted in the urine from 40,000 to 130,000 mouse units of chorionic gonadotropic hormone daily, which was increased up to 440,000 units by injection of pregnancy serum. The excretion of estrone, estradiol, and estriol was observed in quantities greater than in normal menstruating women, and explains the appearance of marked gynecomastia. Extraction of two 48-hour specimens of urine showed 16.5 and 10.5 mgm. of a crystallized hormone thought to be pregnandiol.—H. G. W.

Prostate

BARON, E., and A. ANGRIST. [Queens General Hosp., Jamaica, N. Y.] INCIDENCE OF OCCULT CARCINOMA OF THE PROSTATE AFTER FIFTY YEARS OF AGE. Arch. Path., 32:787-793. 1941.

The incidence of carcinoma of the prostate is high, and higher as determined by the pathologist than by the clinician. By examination of random sections taken from prostates of 364 men over 50 years of age, 9.9% of these prostates were found to contain occult carcinomas, and 4.9% died of extensive prostatic adenocarcinoma with metastases, making a total incidence of 14.8%. In a series of 50 unselected consecutive autopsies on men over 50 years of age the prostate was examined completely and the incidence of carcinoma was 46%, although prostatic carcinoma was not the cause of death in any case. The incidence of occult carcinoma increases with age, as do inflammation and adenomatous hyperplasia, although the lesions could not be correlated. Minjature carcinoma usually occurs in an area of atrophy, and more often

in the lateral lobes or at the junction between the lateral and posterior lobes than in the posterior lobe proper. Because of the high incidence, the small size of the tumors, and the fact that metastases are not found, it must be assumed that the tumors are slow-growing and seldom the cause of death.—H. G. W.

deVRIES, J. K. [New York Hosp., New York, N. Y.] THE DIFFERENTIAL DIAGNOSIS OF CARCINOMA OF THE PROSTATE WITH SKELETAL METASTASES AND OSTEITIS DEFORMANS (PAGET'S DISEASE OF BONE). J. Urol., 46: 981-996. 1941.

A study of 42 cases of prostatic carcinoma with bone metastases, and 17 cases of Paget's disease in men, occurring in 44,000 admissions. By the careful use of the various diagnostic factors, it was possible to make a correct diagnosis in all but 3 cases.—H. G. W.

HUGGINS, C., W. W. SCOTT, and C. V. HODGES. [Univ. of Chicago Clinics, Chicago, Ill.] STUDIES ON PROSTATIC CANCER. III. THE EFFECTS OF FEVER, OF DESOXYCORTICOSTERONE AND OF ESTROGEN ON CLINICAL PATIENTS WITH METASTATIC CARCINOMA OF THE PROSTATE. J. Urol., 46:997-1006. 1941.

Spontaneous febrile infections temporarily reduced the serum phosphatases in advanced carcinoma of the prostate where these enzymes were abnormally elevated. With subsidence of the infection and fever the enzymes returned to their previous high values. Desoxycorticosterone acetate, injected in physiologically large doses, caused only a small reduction in the output of prostatic fluid in dogs and so depressed the male genital complex only slightly. In a series of 12 men with advanced prostatic cancer the acid phosphatase values of the serum following castration did not decrease below 10 units in 4 men. In this resistant group, desoxycorticosterone acetate did not affect the enzyme levels significantly, but stilbestrol in 1 case caused a reduction below the minimum level effected by removal of the testes. The effects of stilbestrol on the acid phosphatase level were clear-cut, and the administration of the estrogen and its discontinuance were repeatedly reflected in alternate depressions and elevations of the acid phosphatase curve.—H. G. W.

HUGGINS, C., R. E. STEVENS, JR., and C. V. HODGES. [Dept. of Surg., Univ. of Chicago, Chicago, Ill.] STUDIES ON PROSTATIC CANCER. Arch. Surg., 43:209-223. 1941.

Twenty-one consecutive patients with demonstrable metastases or local extension of prostatic carcinoma outside the capsule were treated by bilateral orchidectomy. Four patients died within 8 months after the operation. In 2 patients the operation was done too recently to predict the outcome. In 15 cases appreciable improvement occurred and manifested itself by a great decrease in the levels of serum phosphatase in all but 2 cases, an increase in weight, appetite, and red blood count, a decrease in pain, shrinkage of the primary lesion, an increase in density of the metastatic lesions in the roentgenograms, and, in one case, improvement in neurological signs of compression of the cauda equina by metastases. This improvement was greater than that following any other form of treatment, but regression was incomplete. Injections of androgen cause an increase in pain.—G. De B.

MUNGER, A. D. [Lincoln, Nebr.] EXPERIENCES IN THE TREATMENT OF CARCINOMA OF THE PROSTATE WITH IRRADIATION OF THE TESTICLES. J. Urol., 46:1007-1011. 1941.

A study of several cases treated by testicular irradiation with resection seems to indicate that slightly better results were obtained than in those cases treated by resection and x-ray exclusive of the testicular application.—H. G. W.

SUTTON, M. B. [Columbia Univ. Sch. of Med., New York, N. Y.] CREATINE-CREATININE METABOLISM IN OLD PATIENTS WITH BENIGN PROSTATIC ENLARGEMENT. J. Clin. Endocrinol., 1:882-888. 1940.

The study was based on data to the effect that in normal young adult men, administration of exogenous creatine results in an average creatine retention greater than 70%. Six elderly individuals with benign hypertrophy of the prostate and 11 aged men without such hypertrophy gave a similar response upon the administration of exogenous creatine. The author infers that this result would be contrary to that expected if androgens were responsible for benign hypertrophy of the prostate.—J. B. H.

CANCER CONTROL AND PUBLIC HEALTH

LITTLE, C. C. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Maine] PROGRAM FOR RESEARCH ON THE BIOLOGY OF HUMAN CANCER. J. Nat. Cancer Inst., 2:133-137. 1941.

The study of cancer, its development and recognition, and varying factors in relation to carcinogenesis are discussed. Two general types of somatic mutations are already recognized. These are the influence of the internal environmental changes directed by one or more genes and the result of external or environmental sporadic etiological factors which are brought to bear upon cells and groups of cells. The many variable factors in the study of these and other topics related to cancer emphasize the complexities of the disease and the difficulties in its study and analysis.

The problem of human heredity and cancer is emphasized in this article. This problem is admittedly complex and lacks the definiteness and accuracy which allows clear-cut conclusions to be applied to individual human pedigrees and to form the basis for answering individual questions as to the probability of incidence of all but a very few among the multitude of human characteristics or traits. Reasons for this being the case are discussed.

Finally, a proposal is presented for a continuing program of research on the biology of human cancer to be begun at the earliest possible opportunity. Five "typical problems capable of immediate study are briefly mentioned as examples of those which might be investigated."—R. C. R.

MACFARLANE, C. [Woman's Medical College of Penn., Philadelphia, Pa.] PROGRESS REPORT ON EXPERIMENT IN CONTROL OF CANCER OF THE UTERUS. Connecticut M. J., 5:814-816. 1941.

In the 3 years since the inception of biannual pelvic examination, 1,000 women have been examined regularly. In this period, 4 early unsuspected cases of cancer of the uterus were discovered. One hundred nineteen lesions of the cervix which are believed to predispose to the development of cancer also were found and adequately treated. These results reveal the importance of this public health measure.—G. De B.